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# PROBLEMS OF SPACE BIOLOGY

## Volume 1

*Edited by N. M. Sisakyan*

*USSR Academy of Sciences Publishing House*

*Moscow, 1962*

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# PROBLEMS OF SPACE BIOLOGY*

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*Translation of "Problemy kosmicheskoy biologii"

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

EDITORIAL BOARD

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# PREFACE

The last few years have been marked by the outstanding successes of the Soviet Union in mastering cosmic space. The pilots and astronauts Yu. Gagarin, G. Titov, A. Nikolayev and P.Popovich have made the first space flights on spacecraft designed by Soviet scientists, engineers, technicians and workers and made a beginning in the era of the mastering of space by man.

In the overall program of research work a new branch of natural sciences - space biology - will assume a prominent place.

The tasks of this new branch of science include the consideration and studying the biological effects of factors pertaining to space flight, development of biological foundations and principles for ensuring space flights (the safety of) and investigation of the conditions, presence and features of forms of extraterrestrial life.

In this symposium experimental and theoretical works are presented which have been carried out primarily during recent years.

The first part contains theoretical and review papers encompassing the basic problems of space biology and giving a general idea on the results and prospects of these investigations.

In the second part results are presented of experimental investigations carried out under real space-flight conditions on satellites and spacecraft in 1960-1961.

In the third part the results of the biological experiment carried out on the second artificial earth satellite with the dog Layka are summarized, an experiment which provided the foundation for medico-biological investigation in space.

The fourth part contains information on experimental laboratory work as well as work on methods.

Thus, the first volume of "Problems of Space Biology" encompasses the main problems and numerous particular problems which form the subject, the content and the methods applied in this new branch of natural sciences.

The communications presented in this symposium reflect various stages of study of the problem (from the preparation of the dog Layka for the experiment on the second artificial earth satellite up to the results of experiments on satellites and spacecraft). Some of the communications should be considered as only representing preliminary results of investigations which are still continuing.

The authors and editors hope that friendly and constructive criticism by their colleagues will help in compiling the next symposium. They also consider that the information presented in this symposium will prove useful for the further development of space biology and hope that it will arouse the interest of specialists in this field and in various branches of natural sciences.

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PART I

THEORETICAL AND SURVEY ARTICLES

# CERTAIN PROBLEMS OF STUDYING AND MASTERING COSMIC SPACE

N. M. Sisakyan, V. V. Parin, V. N. Chernigovskiy,

V. I. Yazdovskiy

The most important fundamental result of the development of science and technology in our time is the penetration of man into cosmic space. The remarkable flights of the astronauts Yu. Gagarin and G. Titov represent a decisive step in the mastering of the boundless space and resources of the Universe. However, only the first steps have been taken; these have enabled the formulation of the problems and possible methods of solution rather than actually solving the enormous number of scientific and technical problems involved.

What will this contribute to mankind and the future of civilization?

Undoubtedly, scientific concepts on the Universe as a whole, knowledge of the laws of nature and the possibility of controlling nature have immeasurably widened. When we turn to the space of the Universe, and to celestial bodies, we are presented with an incomparable and inexhaustible possibility of future utilization of the energy resources of the Universe. It is difficult to predict to what extent it will be possible to exploit the mineral resources or organic matter of celestial bodies for the benefit of the Earth or future colonies on planets.

Our knowledge of the influence of cosmic factors on terrestrial events and particularly on processes taking place in the atmosphere will become more and more complete. The prospect of accurate weather forecasting is within sight and this will be of very great importance in most fields of human activity and particularly in agriculture.

Creation of automatic scientific stations in space will permit more accurate and larger scale investigations of the processes which take place in the crust of the Earth, the lithosphere, and will also solve practical problems of geomorphology, seismography and geology. Development of radio-astronomy methods will provide a possibility for forecasting periods of increased solar activity, ionosphere disturbances, breaks in radio communication, disturbances in the navigation of ships and will also assist in solving numerous other important practical problems.

Utilization of satellite communications and relay stations opens up extensive prospects in the field of world-wide communications and television. Solution of this problem in the very near future is a real possibility.

Thus, study or cosmic space and investigation of our planet "as seen from a satellite" will permit obtaining tangible results for solving a large number of purely "terrestrial" problems.

In addition, the development of astronautics will be a powerful stimulant to progress in various branches of science and technology, the achievements of which will find extensive applications in many branches of the national economy.

What has been said so far applies not only to achievements to date but also to what can be anticipated in the future. However, we are justified in looking also into the more distant future. The prospect of interplanetary travel is just opening up.

The difficulties to be overcome were understood better by K. E. Tsiolkovskiy than by anyone else; predicting the attack on cosmic space he wrote:

"Undoubtedly success will be achieved but ... any idea that the solutions will be easy will lead to delusion ... many people, now working with enthusiasm would shy away in terror if they were aware of the difficulties of the task.

"However, how wonderful it will be when the aim is achieved. The conquest of the solar system will not only provide energy and life which will be two billion times more abundant than energy and life on Earth but space is still more abundant."

As the great scientist has said, mankind will not remain on Earth forever; a time will come when the epoch of extraterrestrial activity of man will begin.

As long ago as the beginning of this century (1908), K. E. Tsiolkovskiy expressed the idea that after creating an artificial satellite of the Earth, capable of returning to Earth without damage, it will be necessary to solve the biological problems associated with maintaining normal activities of the crews of spaceships. He assumed that then a large number of rocket "settlements" - space stations, would be put into an orbit near the Earth which would be in continuous communication with our planet. All this he considered to be prerequisite for developing the activity of man "outside the atmosphere", which, according to his ideas will occur in several stages:

1. Travel within the limits of the solar system;

2. Settlement of people throughout the entire solar system;

3. Large scale development of industry, science and culture of the citizens of the "Solar fraternity";

4. "The population of the solar system" - wrote Tsiolkovskiy "will reach a hundred thousand million times the present population of the Earth. A limit will be reached after which it will be necessary to populate the entire Milky Way."

How grandiose and daring were the prospects outlined by the founder of astronautics! Many of his scientific predictions have been realized in our time! Can there be any doubt that the space of the Universe, which contains enormous latent possibilities and resources, will belong to mankind?

The achievements of modern science and technology, particularly the outstanding successes in astronautics, have given rise to a new branch of science - space biology.

As in other new fields of knowledge, space biology arose and was formulated at the meeting point of a number of scientific disciplines. Apart from various biological sciences, space biology relies widely on achievements in the fields of physics, chemistry, astronomy, geophysics, aerodynamics, radio-engineering and many others.

On the other hand, the scientists in these disciplines, as well as the engineers and designers, who help in investigating and mastering cosmic space turn with increasing interest to the results of space biology and present new problems at an ever increasing rate.

They are interested in the biological potentialities of the living body, particularly in the physiological potentialities of man as applied to conditions of space flight. It is very important for a designer to know the physiological-hygienic requirements which will have to be met by the systems and structures designed to ensure the necessary living conditions of man in the spaceship or on any celestial body, the requirements to be met by the equipment designed to guarantee safety of flight and the systems for observation and control of spacecraft. The representatives of various branches of natural sciences also expect that space biologists will investigate that mysterious but extremely fascinating field - the study of extraterrestrial life and its properties.

Thus, the first feature of space biology, which distinguishes it from long established biological disciplines, is the inter-relation and the organic connection with other fields of natural and technical sciences.

The second feature of space biology is that it is a young science and, therefore, the number of tasks and the problem of future investigations are considerably wider than the results so far achieved. This is the source of creative ideas, bold research and inventiveness of the scientists who devote themselves to this branch of knowledge.

In spite of the fact that it is a young science, space biology has created and continues to create specific methods of investigation which radically differ from methods currently used in laboratories or biological field investigations. A clear example of this is the large number of methods of biological radiotelemetry (biotelemetry) and new techniques of biological experiments which are realized automatically by means of special instruments in accordance with a predetermined program, and the scientific information obtained is recorded on board the spacecraft or transmitted to ground observation stations.

Practical investigations have shown that experiments realized on spacecraft and satellites produce a very large volume of scientific material, the evaluation of which frequently requires new methods and even new principles for organizing scientific work.

It is increasingly necessary to apply computing techniques, and to design special electronic computers intended for handling the biological information obtained during the flight. The latter are particularly important during manned flights of satellites when the biotelemetry serves not only for research purposes but also presents an extremely important and necessary method of operational medical control. A special communication is devoted to this problem.

It would be difficult to present fully at present the scientific problems which space biology encompasses but we believe that the following three basic problems can be adequately defined:

1. Study of the influence of extremal factors of cosmic space on living organisms of the Earth;

2. Investigation and development of biological foundations for ensuring space flights and life on planets;

3. Study of the forms and conditions of life outside the Earth.

As can be seen, space biology has its own range of specific problems which so far have not been the subject of investigation by any other scientific disciplines, including aviation medicine. For instance, such problems as study of the physiological effect of prolonged weightlessness; the biological effect of cosmic radiation, and the specific damage produced by it; the relative biological efficiency of the cosmic radiation. In the same way the tasks of space biology will

encompass for the first time in the history of science the study of the ability of living beings to survive under extreme conditions of space flight and on other celestial bodies, as well as developing methods that will ensure normal life under such conditions.

It is always useful to judge the trends of scientific investigations from the historical aspect. We believe that it is possible to outline five basic stages of development of space biology in the Soviet Union.

The first stage is linked with defining the problems of space biology on the basis of a scientific prognosis and biological interpretation of the physical properties of the upper layers of the atmosphere, cosmic space as well as the flight characteristics of rockets.

The achievements in natural sciences provided an adequate theoretical foundation and enabled the necessary preparations to be made for performing biological investigations in high-altitude rockets in the early 1950s.

The second stage consisted in conducting experimental investigations under conditions approaching those of space flight. At this stage a large number of biological investigations were carried out in the Soviet Union using high-altitude rockets which reached altitudes of 100, 200 and 450 km above the Earth's surface.

The results of the investigations have proved that animals withstand satisfactorily flight conditions in rockets. No pathological and irreversible changes in the basic physiological functions were observed either during the flight time or after the flight.

It is pointed out that an important advantage of the experiments using rockets is the possibility of studying the effect of a wide range of space flight factors. However, rockets launched vertically or along an inclined trajectory are airborne for only a few tens of minutes. For studying the biological influence of flight factors, however, it is of decisive importance that the flight should be of long enough duration for the reactions of the organism to fully manifest themselves and for an appropriate reconstruction to occur. For instance, it can be assumed that in short duration flights in rockets, basically it is not the influence of weightlessness which is investigated but only the aftereffects of accelerations.

Therefore, from the biological point of view flights along a ballistic trajectory cannot be considered as being space flights in the strict sense. Extremely favorable potentialities for such work have been opened up by using for biological investigations artificial Earth satellites which can be in orbit for a practically unlimited time.

The features of the third stage of investigations are that experimental investigations are carried out under conditions that are similar to space flight from the biological point of view.

The main result of the historically important biological experiment with the second Soviet earth satellite is the proof that a highly organized animal can exist for a long period under conditions of weightlessness. This experience has also shown the correctness of the methods and means used for ensuring the necessary conditions for living in the satellite capsule.

In the fourth stage biological experiments were carried out on spaceships in which the animals were returned to Earth after the flight and then subjected to thorough investigations.

The program of biological investigations in these space flights was considerably extended by using a large number of biological specimens - ranging over a great variety of species.

A series of remarkable biological experiments on the second, third, fourth and fifth satellites solved very many important problems. Basic data were obtained which enabled a decision on a subject of great importance, namely, that manned flight along a circular orbit located considerably below the radiation belts would be safe from the biological and medical points of view and would not affect health and life.

The fifth stage consists of flight of man into space.

Successful completion of the experiments on spaceships, proof of the reliability of operation of the systems for supporting life, control and return to Earth, reliability of the communication and telemetering channels, directly permitted the preparation and realization of manned flight into space.

Outstanding achievements at this stage were the first flights of the Soviet astronauts Yu. Gagarin and G. Titov on the spaceships "Vostok" in orbit around the globe.

This is a brief enumeration of the basic stages of development of space biology in the Soviet Union.

It is pointed out that development of problems of space biology increasingly attracts the attention of many non-Russian scientists and scientific establishments. The scale of these investigations is wide and enjoys the extensive support of organizations who are responsible for planning work relating to the investigation of the Universe.

The meetings of international congresses on aviation and space medicine in Louvain, Rome, London and Paris, as well as symposia carried out by the International Organization for the Study of Cosmic Space (COSPAR) and some sessions of UNESCO, etc. have been devoted to discussing a number of space biology problems.

It can be seen, therefore, that space biology is becoming an internationally acknowledged discipline and the names of numerous major scientists are associated with the development of this science.

One of the important problems of space biology, particularly of space physiology, is the investigation of the influence of extremal flight factors in space on various aspects of the biological activities of a variety of living organisms. On the basis of these investigations the problem of the possible injury to the body by these and other factors (or a complex of factors) is being solved and methods and principles of protection are being worked out.

The space-flight factors which can influence living organisms are sub-divided by the authors of this communication arbitrarily into the following three groups:

1. Factors associated with the flight dynamics of the spacecraft (overloads, vibrations, engine noise, weightlessness);

2. Factors that characterize cosmic space as a distinctive habitat for living organisms (ultraviolet, infrared, visible radiation, ionizing radiation, gas concentration, the peculiar thermal regime, etc.);

3. Factors associated with the relatively long life of an organism (body) under the artificial conditions of a spaceship (isolation, limited space, features of the microclimate, feeding, life rhythm).

The influence of the factors associated with space flight begin to manifest themselves even at the boundaries of the atmosphere at various altitudes from the ground. This is the so-called equivalent of cosmic space and the zone of the atmosphere whether it manifests itself is referred to as the aeropause.

The atmosphere of the Earth provides man and other animals with the necessary conditions for living and protects them reliably from a number of harmful factors of outer space (for instance, cosmic and ultraviolet rays).

It is not possible to describe in detail the physical parameters and the physiological influence of various factors and therefore only a few of these will be briefly outlined. At an altitude of 15,000 m, where the barometric pressure is 87 mm Hg, breathing ceases. At an altitude

of 19,200 m the liquids in warm-blooded animals begin to "boil" since the barometric pressure at this altitude is equal to the pressure of the water vapours in the liquid media of the body at 37°C. At an altitude of 24,000 m it is essential to use regenerative type hermetically sealed capsules. Due to technical, thermodynamic and toxicological conditions, the physiologically necessary barometric pressures can no longer be maintained in the capsule by means of outside air compressed with a compressor.

As regards primary cosmic rays and ultraviolet rays of wavelengths 3000-2100 Å, the layer of the atmosphere above 36,000-40,000 m will be insufficient for absorbing cosmic rays and the layer above 42,000-45,000 m will be insufficient for absorbing ultraviolet rays and, therefore, the injurious biological effects of these rays will begin to manifest themselves. Above 100-120 km from the Earth's surface there is a danger of collision with meteorites. Due to the extreme rarefaction of the atmosphere, sound waves do not propagate, light does not scatter and therefore the conditions of illumination are unusual: sharp contrasts between intensively illuminated and darkened surfaces. The normal perception of space, of depth, is absent. The conditions for adaptation of the retina, the accommodation of the crystal lens and the convergence of the eyeballs are unusual.

It is well known that as the distance from the Earth increases its attraction decreases and thus also the weight of all objects. At an altitude of 6400 km (a distance equaling the Earth's radius), the weight of objects is only a quarter of what it is at the Earth's surface, at an altitude of 12,800 km - 1/9, at an altitude of 57,000 km - 1/100 etc. However, in the spacecraft there will be weightlessness due to the laws governing its movement.

Study of the physiological effect of the state of weightlessness is of very great theoretical and practical interest. This problem has been little studied since under terrestrial conditions the state of weightlessness cannot be simulated for any length of time. Weightlessness can only be studied by means of high-altitude rockets and artificial satellites. "Ground" living has so far not posed this problem to science. The situation is different now....

What effect has disconnection of signalling from a wide range of receptors, the function of which is associated with the gravitational field of the Earth, on the state of the nervous system? What is the influence of the unusual state of the vestibular analyzer on the functioning of other sense organs and their interaction? What are the possibilities of adaptation to these conditions? The inter-relation between the vestibular and oculomotor apparatus and the role of the vestibular analyzer in

visual perceptions are well known. The role of gravitational factors on blood circulation is important. However, most investigators consider that the living body can become acclimatized to these unusual conditions, its great adaptability compensates for the cutting out of the vestibular and the partial cutting out of the skin and proprioceptive reception. In this compensation the visual reception, which corrects the behavior and functioning of man, plays an important role; it transmits to him information on the position of the body in space, on the mutual location of limbs, and on the tension of the muscles required to carry out certain functions.

Available experience indicates that the accelerations required to leave the gravitational field of the Earth, as well as the accelerations associated with re-entry, are fully tolerable.

The most tolerable directions of acceleration are at present being elucidated (for instance, it is known that it is easier to withstand transverse accelerations: front to back or back to front). The following are also being investigated: the most rational position for a man, the position of the individual parts of the body (the angle of inclination of the trunk, degree of bending of the legs); the regime of building up the accelerations, the ratio of the magnitudes and the duration of their effects; the shape and design of the chair, anti-g suit, the system of fixing, etc.

The danger of injurious biological effects caused by cosmic radiation is one of the most difficult problems encountered in mastering cosmic space. The available information on cosmic rays and in particular on its biological effects is inadequate.

The radiation fields around the Earth and in cosmic space are non-uniform. Celestial bodies with their mass and magnetic fields interact with cosmic radiation and the moving masses of charged particles (plasma) bring about a very complicated radiation field pattern which changes with time and space.

During recent years, Soviet and American scientists have discovered, by means of artificial earth satellites and space rockets, the existence of powerful radiation belts around the Earth which are of great importance from the biological point of view.

Determination of the relative biological effect of cosmic radiation is an extremely complicated problem, firstly because it is not possible to reproduce on Earth the high-energy particles of cosmic radiation and, secondly, because the magnitudes of the relative biological effectiveness of individual types of radiation differ depending on the biological index being observed (for instance, the reaction of the blood-forming system or the production of cataract in the eye).

The biological effects of corpuscular types of radiation have their own specific features: their effect may well be cumulative and irreversible, they have a considerable genetic effect, etc. Of great importance is the study of the genetic influence of cosmic radiation. For génetic investigations special biological specimens are required. Use of minute specimens in genetic studies (drosophilae, actinomyces, bacteria, etc.) allows a great deal of statistical material to be collected for detection of the genetic biological effect which may result from small radiation doses. Several papers of this symposium are devoted to this problem.

Taking into consideration the discrete nature of cosmic radiation, it can be assumed that the biological effect of individual particles will be detected in a number of cases more easily on minute biological objects (for instance bacteria) than in a multicellular organism.

The problem of the biological effect of cosmic radiation has interesting aspects from the general biological point of view, namely, with respect to elucidating the existence of forms of life in cosmic space (spore forms of microorganisms), on the possibility of carrying them from one celestial body to another, and on the origin of life on celestial bodies, etc.

As long ago as 1934 at the Conference on the Study of the Stratosphere, a large number of important problems concerning the study of the biological effect of cosmic radiation were posed; this can be considered as a great credit to Soviet science.

In the work of the conference such eminent scientists as S. I. Vavilov, L. A. Orbeli, V. I. Vernadskiy and others participated.

As regards general problems of space biology, some should be specifically mentioned which relate to the physiology of the higher nervous activity and the psychology of man.

Remaining in an isolated capsule of a limited volume over a long period imposes on man considerable psychological problems which need to be seriously studied and rational regimes of work and rest have to be worked out. The man will to some extent be deprived of his accustomed social medium, a number of exteroceptive and interoceptive stimuli. Under conditions of weightlessness the proprioceptive and interoceptive stimuli will be appreciably limited. All this, together with disturbance of the accustomed rhythm of life (for instance, the change from day to night, from work to rest), may bring about functional and vegetative disorders if appropriate measures are not taken in regard, particularly, to physical exercise. It is emphasized that the feasibility of long duration space flights will be determined primarily by the success achieved in evolving a system for supporting the life of the crew which would not

rely on large reserves of food, water and oxygen. The problem can be solved only by reproducing (or simulating) the natural material-energy relations between the human body and nature on Earth, effected by the biological closed circulation of matter based on utilizing the energy of the Sun. Essentially, the provision of living conditions for the crew reduces to establishment of animal and plant societies, including man, which will be able to withstand for a sufficiently long time the peculiarities of space-flight conditions and satisfy the material and energy requirements of the crew. In organizing more or less prolonged flights it is necessary to strictly limit the weight and size of the spacecraft. Preparation of special highly-concentrated tablets and briquettes containing all the required food substances and vitamins cannot be considered a satisfactory solution of the problem, since the intestines require a certain volume of food and there are also other physiological features in man and animals which will probably not permit living on such a diet for a long time. Furthermore, when travelling to distant planets it will be impossible to ensure a sufficient reserve of foodstuffs even in the form of concentrates.

For this purpose many scientists propose and work intensively on methods allowing the creation of a closed cycle of substances in the capsule of the spaceship. It is proposed to utilize continuously the water from the discharged urine after subjecting it to various methods of purification: distillation, freezing, electro-osmosis, absorption, ion-exchange processes; various methods of utilizing solar energy in this process have also been developed. From the point of view of economy of weight, size and electricity consumption, each of the enumerated methods has certain disadvantages and advantages and can be used for a certain flight duration. Various methods of complex chemical processing of faeces are also proposed, the aim of which is to utilize in the fullest possible way the energy of all available substances for food.

However, the most promising will obviously be biological methods associated with growing on faeces various plants which could subsequently be used as food. Simultaneously, these plants would be used for regenerating the air in the cabin, since they absorb carbon dioxide exhaled by man and animals and enrich the atmosphere of the capsule with oxygen. Thus, the natural circulation of substances and gases in nature will be imitated on the spaceship. Of particular interest in this respect are certain water plants which grow very fast, increase their mass to many times the original mass per day and contain almost all the necessary food substances.

The biological and biochemical characteristics of some of these plants are presented in the respective communications of this symposium. Since a relatively great weight and volume are involved, biological methods of regeneration of gases for the breathing air of the crew can be economical and rational only in the case of flights of sufficiently long duration.

Chemical methods of air-regeneration can be used for shorter flights. As is well known, such a method has been used in biological experiments on the second artificial Earth satellite.

In conclusion, it is necessary to dwell on a major section of space biology - exobiology, the aim of which is to study the presence and features of forms of life outside the Earth - in space, on planets of the solar system.

To many people, the various hypotheses on the existence of living matter in the Universe are well known. Renowned scientists have also expressed their deep conviction that life does not exist on our planet alone. However, the fundamental problem is finally to find strict scientific, irrefutable proof that this is true.

All the attempts to solve the problem of the existence of life, e. g., on Mars by observation from the ground involve major difficulties. The distance that separates us from that planet is enormous but, unfortunately, the main difficulty consists in the fact that observation is impeded by two atmospheres: our own - the atmosphere of the Earth, and the atmosphere of Mars. This fact makes difficult and limits the possibility of applying optical instruments. It can be assumed that major successes will be achieved by instruments fitted on board space stations and rockets and not on Earth. However, observatories on Earth have carried out very successful work. It is sufficient to mention the work of Soviet and foreign astronomers. By means of accurate instruments they were able to detect in the dark regions of Mars the so-called "seas" and absorption spectra, which are very characteristic for organic compounds of biological origin.

If we add to this that in meteorites hydrocarbons as well as some organic compounds were discovered which were closely connected to terrestrial organisms, it becomes evident that much work has still to be done in this direction using "terrestrial means." Of first importance at present is the task of detecting and investigating micro-organisms, spores and the elementary organic substances in cosmic space.

At present, it is possible not only to pose but also to try and solve experimentally the problem of the validity of the trans-sperm theory of Svante Arrhenius.

It is possible to assume that conditions of cosmic space will not necessarily cause destruction of the simplest representatives of the plant and animal kingdoms of the Earth. With the development of space flights the problem of preventing uncontrolled contamination of the Universe with terrestrial forms of life as well as bringing extra-terrestrial forms of life to our planet is of great importance.

To biologists the prospect of comparing forms of life detected in cosmic space with forms of terrestrial life is extremely alluring. This would permit elucidation of the nature, the method of formation and evolution of living matter in the Universe and confirm generally valid laws of development of matter.

The Universe is boundless and for us the number of tasks facing science is also boundless.

These tasks are waiting to be undertaken.

The result will be the discovery of new secrets of nature, the strengthening of the power of Man over it, the advancement of civilization and the happiness of future generations.

SUMMARY

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The evolution of space biology and its main problems are briefly discussed under three headings: a) factors due to the intrinsic condition of flight dynamics of the space vehicle (e.g. accelerations, vibrations, weightlessness); b) factors due to the nature of the ambient space (e.g. electromagnetic and corpuscular radiations, composition and thermal state of space); c) factors created by the exposure of human beings to the artificial environment of the space ship (isolation, confinement to restricted space, different diet and different rhythm of life). The importance of the more fundamental and general aspects of space biology relating to the evolution of life and the possibility of transplanting various forms of life from one celestial body to another are also briefly discussed.

Author

Translator's note: This is an original abstract and not a copy of the English "summary" printed with the article. ~~Author~~

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PROBLEMS OF SPACE BIOLOGY

N. M. Sisakyan, O. G. Gazenko, A. M. Genin

Recent successes in science and technology permit a planned, systematic study and mastering of cosmic space.

Wide and extremely fascinating prospects of investigation are opening up for biology which reduce to the following three fundamental problems: the influence of extremal factors in space on the Earth's flora and fauna; the biological conditions for ensuring successful human space flights and the maintenance of life on planets; the modes and conditions of extraterrestrial life.

In view of the fruitfulness of convergent methods of investigation, it is to be hoped that even a brief presentation of these problems will attract the attention and be of interest to workers in the different branches of biology, the cooperation of whom is necessary for the successful formulation and solution of the problems involved in space biology.

## Extremal Factors in Cosmic Space

Biological space investigations will be concerned with many aspects and methods of biology which are of fundamental importance. Organisms on Earth became adapted to their conditions of existence during their evolution (influence of the gravitational field, the barometric pressure, the background of ionizing radiation, etc.). Cosmic space is a medium the conditions of which differ radically from those encountered by living organisms within the limits of the biosphere of the Earth. The negligible density of matter, the absence of molecular oxygen, the presence of intensive and biologically very active radiations, the peculiarity of the temperature regime - all these exclude the possibility of survival of highly organized specimens of the Earth's living creatures in free space.

Protection of man and his possible companions (animals and plants) from the unfavorable, possibly damaging, influence of extremal factors of flight in space presents a very complex and difficult biotechnological problem.

Two factors require particular attention: the biological effect of cosmic ionizing radiation and the state of weightlessness.

Danger from the injurious biological effects of cosmic radiation is unfortunately one of the main difficulties encountered in mastering cosmic space. From the radiation point of view cosmic space is nonuniform. In the neighborhood of the Earth the existence was discovered of belts

of increased radiation, whose intensity reaches levels which are dangerous to living organisms. It is fully possible that analogous or even more intensive radiation belts exist in the neighborhood of other planets which have magnetic fields. Furthermore, it must be borne in mind that the intensity of radiation in cosmic space and its qualitative composition is subject to continuous fluctuations with time, depending on a number of causes. In this respect sun flares require particular attention; during the period of sun flares the radiation intensity may increase by several orders of magnitude and present a very great danger to living organisms. Unfortunately, it is very difficult to make any long-term forecast of sun flares.

In studying the biological effects of cosmic radiation only the first steps have been taken and the problem requires further careful study. It is necessary to elucidate the relative biological effectiveness and the characteristics of the effect of heavy nuclei that can bring about a very high ionization density. Also of considerable importance is the combined effect of ionizing radiation (particularly of cosmic radiation) and of other flight factors, for instance, those of acceleration and vibration. Changes in the radiation sensitivity of biological material (for instance, genetic effects), can be anticipated as well as changes in the resistance of the organisms to the effect of other factors.

The belts of high radiation intensity which were discovered in space impose on biologists important practical tasks relating to the protection of space crews.

Undoubtedly, one of the most difficult problems is protection against the protons of the inner radiation belt, the lower boundary of which is located approximately 600 km above the surface of the Earth in the region of the equator.

By correct plotting of flight trajectories it should be possible to by-pass zones of high-intensity radiation, but investigations into developing physical, chemical and biological means of protection are also very promising.

The state of weightlessness is one of the characteristic factors of space flight which has been very little studied. It may be possible to remove the difficulties caused by weightlessness by creating artificial gravity in the spaceship. To what extent this is necessary and the magnitude of the artificial gravity to be recommended requires more experiments to be carried out.

Biological experiments so far carried out on spacecraft indicate that for a matter of days, or an even longer duration, the state of weightlessness will have no adverse effect on the physiology of living

matter. Moreover, it is reasonable to hope that the coordination of movements and the orientation of man in space will not be impeded by weightlessness.[1] What has not been clarified at all is whether a long stay in space in a state of weightlessness will so change the functional state of organisms that on return to their normal gravitational field they will behave as though they are being subjected to excessive gravity loads.

It should also be borne in mind that the gravitational field of the Earth undoubtedly influences the physiology of cellular and subcellular structures, particularly during embryogenesis. It is assumed that during the first stages of division of a fertilized egg, the axis of the blastula must have a definite orientation in the gravitational field. How these processes will proceed in the absence of gravity presents an interesting biological problem and will be of importance in future space flights.

Of all the factors which living organisms may encounter during space flight the problems of cosmic radiation and weightlessness are the most difficult to solve. The total number of extremal factors is considerable and we cannot be sure that they are all fully known. It is quite possible that further investigations of the physics of cosmic space, combined with biological experiments will reveal additional hitherto unknown conditions or biological effects to which, at present, no importance is attached.

## Biological Bases for Ensuring Space Flights

To compare specific concepts on the various means of ensuring long duration space flights, it is necessary to formulate the basic requirements and data to which these systems must correspond.

Such a formulation will be both complicated and controversial.

It is obvious that space flight will bring discomfort to the crew in one way or another since the very extensive and many-sided necessities of man will inevitably be in contradiction to technical possibilities. The problem is to determine the limit of permissible discomfort and to find a reasonable compromise between the possibilities which can be realized in the near future and the essential requirements for man. A complete and general solution for this problem is hardly possible. However, even now it is necessary to formulate a certain approach to some of the

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1 Valuable data obtained during the group space flight of A. Nikolayev and P. Popovich confirm the assumption expressed here.

main problems, keeping in mind the fact that subsequent investigations and experience during actual space flights will introduce important corrections to the original approach as well as suggesting more up-to-date concepts.

The important problem of ensuring the life of astronauts is the development of methods of feeding. We possess sufficiently extensive material to determine the optimum ratio of the individual foodstuffs to be included in an astronaut's rations. The necessary mineral composition of the food has been investigated in sufficient detail and the quantitative requirements of most of the vitamins have been established. These requirements have been established for various conditions of professional activity. However, up to now it has not been possible to carry out investigations which would allow formulation of the food requirements of astronauts.

These requirements have only been tentatively worked out and are justified only because in our view there is no direct reason to assume the existence of any important features in the metabolism of men who perform long duration space flights. Assuming that on a spaceship a man would carry out light or medium heavy work, we can tentatively take as a basis for his food requirements the standard of feeding required for similar conditions on Earth. On this basis the daily ration of the astronaut would be as follows:

1. Calorific value - 3000 kcal.

2. Composition of the food: a - proteins - 110 g, of which 63 g should be animal proteins; b - fats - 90 g; c - carbohydrates - 418 g; d - mineral substances and vitamins - 22 g.

3. Water - 2200 g.

The composition of the daily ration may vary, particularly with respect to fats and carbohydrates (provided the total calorific value of the food is maintained), but it would hardly be possible to make important changes in the ratio of the foodstuffs without careful and extensive investigation. Thus, the total weight of the daily food ration should be 640 g of assimilable foodstuffs and about 2200 g of water.

Of great importance from the point of view of satisfactory feeding is also the quality of the food, particularly the taste, consistency and methods of preparation, etc.

The individual peculiarities of man, his habits and inclinations cannot be ignored when working out the food rations for feeding astronauts in flight for many years. Under monotonous flight conditions with limited information and a sharp drop in stimuli and impressions, varied

and tasty food could be of considerable importance in stimulating the mental and physical tone of the body. Therefore, in developing systems of ensuring space flights of long duration it is advisable to get the food ration of a man as near as possible to the optimum. Assuming that the proposed food ration fully covers the energy losses of the astronauts, and that during the flight there will be no continuous increase in body weight or any appreciable transformation in the biochemical structure of the organs and tissues, the gas exchanges of man can be calculated with sufficient accuracy.

Thus, for oxidation of the food ration, 632.8 liters (882.25 g) of oxygen will be required and some 566.5 liters (1121.67 g) of carbon dioxide will be exhaled (breathing coefficient 0.895) with about 340 g of water. The quoted figures may fluctuate periodically with time, but over a period of time, these fluctuations would be levelled out.

Thus, for ensuring the life and working performance of man during space flight he will require daily 640 g of fully assimilable food (dry weight), 2200 g of water and 882 g of oxygen.

Consequently, for a three-year space flight a five-man crew would require a reserve of oxygen, water and food of no less than 19 tons. The actual figure will be considerably higher due to the weight of the packaging materials, special storage facilities, control equipment, etc.

The creation of partial or full circulation of substances in the space capsule could considerably reduce this weight and, what is particularly important, reduce the dependence of the necessary weight on the duration of the space flight. Such a closed cycle of chemical substances is fully possible with the utilization of the radiation energy of the Sun.

The greatest effect can be obtained by achieving a full cycle of the water. Solution of this problem involves the least difficulty and does not require complicated or cumbersome equipment. Various physical and physico-chemical methods are fully applicable (distillation at normal or at reduced pressures, electro-osmosis, purification by means of ion-exchange resins, etc.). Since the weight of the supply of drinking water is 2200 g per man per day, the gain is quite obvious.

Much greater difficulties will be encountered in achieving a closed cycle of the oxygen. However, this problem can also be solved by applying physical and biological methods. Of great interest is the photolytic decomposition of carbon dioxide with hard ultraviolet radiation on a copper or other catalyst. Also of considerable promise are investigations in the field of electrolysis (or pyrolysis) of water with subsequent interaction between hydrogen and carbon dioxide. Under certain conditions oxygen may form as a result of this reaction. The initial substances of

this reaction (gaseous carbonic acid and water) are the end products of metabolism and the quantity of oxygen which can be extracted exceeds the requirements of man and, therefore, regeneration of oxygen by physico-chemical methods can proceed according to a closed cycle.

In this respect the investigation of biochemical transformations that occurs in a large number of anaerobic bacteria which result in absorption of hydrogen and gaseous carbonic acid and a release of oxygen is very promising.

Thus, by bringing about a circulation of oxygen in the hermetic capsules of spaceships the problems of long duration space flights, including flights to the nearest planets of the solar system, could be more easily solved.

However, this does not fully solve the problem of the autonomous existence of man in space since the maximum duration of space flight is limited by the food reserves on board the spaceship.

The fullest solution of the problem would be by materializing the idea of Tsiolkovskiy on creating a closed ecological medium in the capsules of spaceships, interplanetary stations and special structures on planets. A decisive link in realizing this idea would be the complete regeneration of food and the fullest possible utilization of the waste products of man.

Theoretically it is possible to visualize the solution of the problem of feeding astronauts by using an artificial synthesis of carbohydrates, fats and amino acids from the carbonic acid (gas), water, ammonia, urine and other end products of metabolism. Formaldehyde, methane and other substances which form during extraction of oxygen from water or carbonic acid (gas) are intermediate links in these reactions. More promising in this respect is the chemical synthesis of the necessary precursors of the basic food substances and their subsequent assimilation by micro-organisms or by other types of biosynthesis, then the biosynthetic products could easily be extracted and utilized as full-value food for the astronauts.

Research in this field is extremely interesting and promising. It goes beyond the framework of space biology and it is undoubtedly of great and wide importance.

However, we must consider the difficulties involved in completing these tasks. As it is unlikely that in the next few years it will be possible to achieve a synthesis of food products from inorganic substances, the most realistic view is the creation of a closed cycle of substances, by reproducing on a small scale the basic principles which occur in the reaction between man and his environment on Earth.

The task consists primarily in selecting from the complex system those natural inter-relations which can be utilized most rationally within the confined space of a spaceship capsule under specific space flight conditions. Of greatest interest in this respect is the synthesis of food by green plants which enables the further synthesis of organic substances to occur in man and animals.

At present the attention of biologists is greatly attracted to utilizing the photosynthetic activities of single-cell water plants or algae for regenerating air and food. The advantage of using single-cell algae stems from the high coefficient of utilization by these plants of the Sun's energy, and the possibility of achieving a relatively constant speed of accumulation of organic substances from small volumes of algal suspension, which is ensured by their short vegetational period and the enormous numbers of individuals all at various stages of development.

It is also necessary to bear in mind that practically the entire biological mass of single-cell algae can be utilized for the feeding of man. Other technical advantages of using algae are also obvious (the possibility of the most rational use of the space of the capsule, uniform distribution of light, etc.).

To create a feeding system which accurately fulfills the physiological requirements of the human body it is necessary to carry out investigations directed to elucidating the possibilities of including higher plants and animals into the closed cycle of the substances in the spaceship capsule. To a considerable extent this would enable the dietary regime of the space pilot to be matched with his natural terrestrial diet. The solution of this problem will undoubtedly require the efforts of a large number of research teams. The need for such investigations is very obvious.

Considering the prospects of future flights, one has to think also of the problem of what will have to be done if during the flight special conditions arise which subject the crew of the spaceship to any type of external effects which may endanger their lives or if a catastrophic shortage of oxygen, water or food should arise.

A possible solution to this problem would be to slow man's metabolism and so bring him into a near anaerobic state. The search for and development of a method for creating at the right time a controlled state approaching the anaerobic or lethargic state is a complex, interesting and extremely important piece of scientific work.

The tasks facing space biologists cannot be resolved without developing the means for providing accurate information of the physiological indices of the body. The rapid progress in the fields associated with cosmic biology (automation, radio-electronics, cybernetics and other related branches of science) provides favorable conditions for this.

In contrast to investigations carried out by biologists on the ground, any measurements in flight have to be transmitted by means of radiotelemetry channels. Biological telemetry thus becomes a fundamental method of investigation as well as a means of monitoring the progress of space flight with time.

Utilization of radiotelemetry introduces a specific distinctive feature into the methods and techniques used in biological experiments. The basic task of biologists and engineers working in this field is the development of new transducers which will transform physiological and biological changes in the body into electrical pulses, the development of automatic systems for operational control of biological material and astronauts during flight, and the further application of computer techniques for data processing.

With increasing duration and range of spaçe flights and because of the anticipated drop in the available capacity of radio-channels, it will be necessary to process automatically in the spaceship itself the biological information and to feed the generalized results not only to the telemetry channel but also to the control system of the spaceship. This will involve developing methods of coding the information, working out rational algorithms for analyzing it and developing small-size economic and reliable digital computers which will permit solution of the medical and biological problems which arise, and thus ensure the safety of space flights.

## Exobiology

The tasks of exobiology include searching for living matter and organic substances in cosmic space and studying the peculiarities of these forms of extraterrestrial life.

The various hypotheses of the existence of life on other celestial bodies of the solar system and in the Universe as a whole are sufficiently widely known. Unfortunately accurate information on this subject is very limited.

A further development of V. I. Vernadskiy's conception of the biosphere is the idea that there is a zone of life within the limits of the solar system - the exosphere of the Sun. On the assumption that organic life, which is based on carbon compounds, may exist in the temperature range +80 to -70°C, it is possible to distinguish an area in space located within the limits of the distance between 92 million and 275 million km from the Sun. There are three planets in the zone, Venus, Earth and Mars, wherein the Earth is located almost at the thermal center of the

exosphere. Its average annual temperature is about $+14^{\circ}C$, while the average temperature of Venus is about $+50^{\circ}C$ and Mars about $-50^{\circ}C$. (Translator's note: Obviously recent American results were not known at the time of writing this book.)

All attempts at solving the problem of the existence of life on Mars by observation from Earth involve great difficulties. Only recently, by means of infrared spectroscopy, absorption spectra were obtained of the dark regions of the planet (the so-called seas), which are considered characteristic of organic compounds of biological origin. Obviously direct proof of the existence of life on this planet, and particularly the investigation of the features of this life, will only be possible when direct contact is made with the subject under study.

In recent years increasing attention has been paid to the investigation of the composition of carbon meteorites. It has been proved that the major part of these meteorites is soluble in organic solvents and contains a high percentage of carbon and oxygen. Mass spectrographic analysis of the carbonaceous substance of the Orgeyl meteorite revealed carbohydrates which are very near to those present in living organisms. This work provides a basis for the hypothesis of the existence of extraterrestrial biogenic processes.

Also, there have been reports that organic compounds and microscopic organisms of extraterrestrial origin were discovered in the depths of the so-called Murray (Australia) meteorite.

In evaluating similar finds it is obviously necessary to be both careful and cautious. However, such information deserves very careful attention and, what is most important, it points to possible ways of carrying out exobiological investigations.

The successes gained in space travel and the prospects of creating scientific space stations open up new prospects for exobiological research. In the first instance it is necessary to verify the presence in space of simple forms of life, their elementary biochemical processes and their subtrates, and how they resemble or differ from those encountered on Earth.

So far, only hypotheses have been put forward on this subject and of these the transsperm theory of Svante Arrhenius is the most popular.

Of all the arguments against the possibility of transferring life from one celestial body to another the following two are the most important: the injurious effect of radiation, and the absence of an obvious natural mechanism which would assist even such small particles as the

spores of microorganisms to overcome the gravitational forces acting at the surface of every planet.

However, the results of certain laboratory experiments permit the assumption that some very stable spore forms of microorganisms may exist in space which are so resistant that they can survive when being carried from one celestial body to another as a part of meteor particles.

Extraterrestrial micro-organisms may adapt themselves to the unusual conditions of space by the development of remarkable mechanisms, brought about by a drastic change in the interaction occurring between them and the surrounding medium. For this reason there is a considerable interest in the possibility of studying the means by which terrestrial forms of life adapt themselves to the new conditions on other planets. It has been shown experimentally that some anaerobic and facultative anaerobic micro-organisms can adapt themselves to conditions which are assumed to exist on Mars.

It is of great interest to investigate the problem of the extent to which these conditions can be withstood by certain plants, particularly lichen and moss.

It is possible that conditions in space may not necessarily lead to the destruction of simple representatives of the organic world of the Earth and still less to the destruction of organic substances. Thus, the following extremely theoretical and practical problem arises: it is necessary to prevent the uncontrolled carrying of terrestrial forms of life and organic matter to other celestial bodies as well as to prevent bringing other forms of life back to Earth. To biologists the prospect is particularly tempting to compare the forms of extraterrestrial life or biogenic products with terrestrial ones. Such a comparison would perhaps enable us to understand the nature of the formation and development of life in the Universe and to confirm the unity of the laws governing the development of living matter.

It can easily be seen that the development of space biology will not be exclusively concerned with interplanetary travel and the conquest of the Universe by man. Space biology may also be responsible for the evolution of new biological concepts which are generally valid and relate to the problem of life as a whole.

SUMMARY

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A brief and general discussion of the effects of radiation encountered in outer space and particularly of that associated with the lower belts of radiation surrounding the Earth. Protection from radiation present in the zone starting at 600 km altitude and comprising primarily

protons is considered particularly difficult. On the other hand, exposure to weightlessness is considered to be relatively safe but two important points are made, namely, the need to study the effects of weightlessness on return to the gravitational field after prolonged exposure to weightlessness and the effect of weightlessness on embryological development. Considerable emphasis is given to the problem of suitable diet during space flight. Three thousand kcal is recommended as a daily ration, including 110 g of albumins of which 63 g should be of animal origin, 90 g of fats, 418 g of carbohydrates and 22 g of mineral substances and vitamins. Two thousand two hundred g of water per day is recommended, this being by far the heaviest part of the daily ration. In view of the heavy weight demand various methods of partial and complete recirculation of food and water are suggested. These include distillation ion exchange and electro-osmosis. Recirculation of oxygen is also briefly described: photolytic decomposition of carbon dioxide with hard ultraviolet in the presence of copper catalysts is suggested. Biochemical methods based on the action of anaerobic bacteria are also discussed. A number of suggestions are made regarding the synthesis of food. These are based on the use of green plants, algae, etc. All these methods aim at converting end products of metabolism into original food. The article is concluded with a brief review of arguments in favor of the existence of organic life in the exosphere. The most suitable region for the existence of organic life based on carbon compounds is between 92 million to 275 million km from the Sun. The importance of allied sciences such as cybernetics, electronics and automation to the rapid development of space biology and the significance of exobiology from the point of view of fundamental biology and methodology are particularly stressed. Author

Translator's note: This is an original abstract and not a copy of the English "summary" printed with the article.

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# RADIOBIOLOGICAL PROBLEMS OF SPACE FLIGHTS

A. A. Gyurdzhian

The flight of man into space must be reliably supported by ensuring satisfactory conditions of life and safety. The danger caused by the biological effects of cosmic radiation is one of the most complex biological problems in the conquest of space.

## Basic Information on Cosmic Radiation

The primary cosmic radiation which penetrates to the Earth from interplanetary space consists of positively charged particles - the nuclei of various elements. They have an enormous velocity, reaching the velocity of light. Regardless of differences in atomic weight and number, the velocity of the various nuclei is about the same. The energy of the particles is basically within the limits of $10^9$ - $10^{18}$ electron volts (ev). However, the content of low-energy particles is relatively higher than that of all the high-energy particles. Therefore, the average energy of particles per nucleon in the upper layers of the atmosphere is only $3 \cdot 10^9$ to $4 \cdot 10^9$ ev. The most powerful up-to-date accelerators can accelerate particles only to velocities of the order of $10^{10}$ ev (for instance, the synchrophasotron in Dubna). Apparently, cosmic-ray particles can exist for a very long time ($10^6$ - $10^9$ years).

The cosmic rays contain protons (hydrogen nuclei), $\alpha$-particles (helium nuclei) and nuclei of the following groups of elements (in order of increasing atomic number): Li-Be-B, C-N-O, Na-Mg-Al-Si, S-Ar-Co and others up to nuclei of Fe and, possibly, of heavier elements. Lighter nuclei are more frequent than heavier nuclei. Thus, according to certain data (Bradt and Peters, 1950 and others), protons comprise 80% of all nuclei, $\alpha$-particles - 19% (according to other data, protons - 84%, $\alpha$-particles - 15%), while all the remaining heavier nuclei form only about 1% of the cosmic radiation. According to the observations of L.V. Kurnosova and others (1960), it was discovered by means of artificial satellites that the ratio of the three groups of nuclei with atomic numbers larger than 2,5,15 ($Z \geqq 2$, $Z \geqq 5$ and $Z \geqq 15$) is in the region of 1000:75:3. The predominance of light nuclei over heavy nuclei is in relatively good correspondence with the distribution of the elements on Earth and in the Universe. However, there are certain exceptions to this rule (for instance the group Li-Be-B). These exceptions are of considerable theoretical interest.

So far, experimental data have not confirmed the presence in cosmic rays of electrons and $\gamma$-quanta in considerable quantities (which was assumed earlier). The possibility cannot be ruled out that cosmic rays contain electromagnetic radiations (of ultra-high frequency and of other types) of lower frequencies than X-rays. These may bring about thermal or other biological effects (Tobias, 1959, and others). However, their ratio (intensity) in cosmic rays is undoubtedly lower by several orders of magnitude than that of charged particles.

The problems of the origin of the charged particles of cosmic rays, their composition, their mechanisms of formation and acceleration, the duration of their existence and their subsequent transformations are very acute in modern physics and is the subject of intensive study (Dobrotin, 1954; Rossi, 1955; Pervichnoye kosmicheskoye izlucheniye (Primary Cosmic Radiation), 1956; Trudy Mezhdunarodnoye konferentsii po kosmicheskim lucham (Transactions of the International Conference on Cosmic Rays), 1959, 1960a, 1960b; Kurnosova and others, 1958, 1960; Vernov and others, 1957, 1958, 1959, 1960; Van Allen, 1960; Shepherd, 1953 and others).

Within the limits of the solar system, cosmic rays are enriched by radiation of solar origin. The sun radiates (in addition to visible and infrared light) ultraviolet and X-rays, electrons, protons, possibly $\alpha$-particles and multicharged nuclei as well as other corpuscular radiation, atoms, nuclei in which the electron shell is partly conserved (Krasovskiy and others, 1958; Trudy Konferentsii po issledovaniyu Solitsa (Transactions of the Conference on Investigating the Sun), 1957; Ellison, 1959; Burnight, 1952; Mandel'shtam, Tindo and others, 1961, and others).

The primary cosmic radiation interacts with the magnetic field of the Earth and with the Earth's atmosphere, is subjected to a variety of multistage transformations and hits the Earth's surface in a very attenuated form.

Primary cosmic radiation is encountered only at an altitude in excess of 35-40 km. Shepherd (1957) considers that the protons of the primary cosmic rays can be detected up to an altitude of 12 km above the ground surface. At sea level the secondary elements of cosmic radiation have only 2% of the energy of the primary particles (Tobias, 1959). The secondary particles which arrive at the surface of the Earth can be produced only by those primary particles which possess very high energies. Therefore, the enormous fluctuations in the intensity of cosmic rays, observed at the upper limit of the atmosphere and caused primarily by the low-energy particles, are observed only to a very slight extent at ground level.

The secondary cosmic radiation, observed at ground level, contains a great variety of components: electrons, positrons, various types of mesons, $\gamma$-quanta, fast and superfast neutrons and protons. In the cosmic

radiation recorded at sea level we can distinguish soft and hard components. The soft, or low-penetration components (electrons, positrons, $\gamma$-quanta, slow particles), are those which can be absorbed by a layer of lead 8-10 cm thick. The hard components (mesons, superfast particles) penetrate deep into the ground (particularly $\mu^{\pm}$ mesons). Relatively slow particles possess a high ionizing ability. At sea level the hard components predominate (70%), while at an altitude of 4.5-5 km the soft components begin to predominate (62%). Radiation from radioactive formations on the Earth can only be detected near to the surface. At the ground surface, about 1/5-1/3 of the background radiation is due to cosmic rays and its relative importance increases rapidly with altitude.

The neutrons that are formed as a result of primary transformations of cosmic rays play a very important role as regards their ionizing effect. The number of neutrons at an altitude of 10-20 km from the surface of the ground ($51^{\circ}$ geomagnetic latitude) is about 7.1 per 1 $cm^2$ sec, while their average energy is assumed at approximately 5 Mev. After colliding with the nuclei of the medium, the neutrons will split the nuclei and recoil nuclei will form. As a result of the passage of cosmic-ray particles through a medium, particularly one containing hydrogen, the number of slow neutrons will increase (fast neutrons will be transformed into slow ones), for instance, passage through a 2.5 cm thick layer of paraffin brings about a thirteen-fold increase in the number of slow neutrons. As a result of this, the maximum ionizing effect of neutrons is considered to be at a level corresponding to a barometric pressure of about 0.1 $kg/cm^2$ (Tobias, 1956).

One of the important characteristics of cosmic radiation in the atmosphere is its ionizing effect. At sea level the ionization of the air corresponds, on the average, to the effect of radiation of an intensity of about 0.1 mrep per 24 hours and consists of about two pairs of ions per 1 $cm^3$ of air per 1 sec. At an altitude of 22-23 km, maximum ionization corresponding to 15 mrep per 24 hours was observed (Figures 1,2). At greater altitudes the degree of ionization decreases and reaches 9 mrep/24 hours. Then this level increases again as a result of the decrease of the screening effect of the Earth at higher altitudes (cosmic rays may impinge both from the top and the bottom hemispheres). Then, at even greater heights (from 500-1000 km onwards), the intensity of the radiation increases sharply, which is associated with the recently discovered radiation belts surrounding the Earth. Beyond the limits of these belts (at a distance of 10-20 Earth radii from the Earth), the intensity of radiation again equals 9-10 mrep/24 hours.

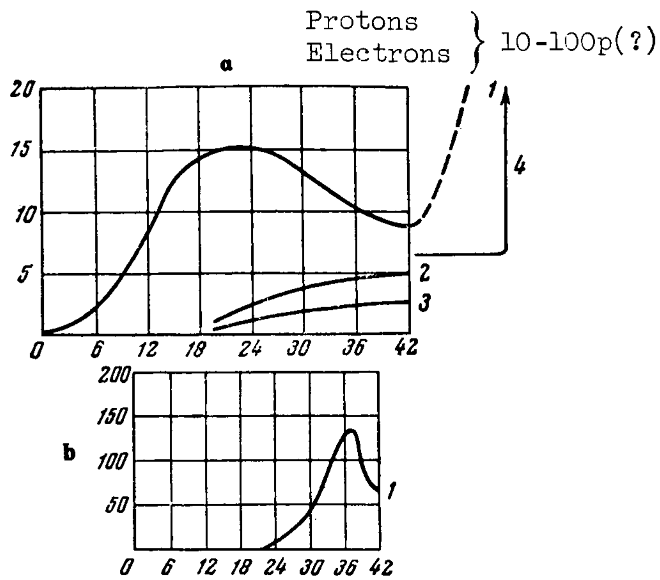


Figure 1. Ionizing Power of Cosmic Rays at Various Altitudes from the Ground. Plot a - along the ordinate - millirep/24 hours, along the abscissa -

altitude, km; 1 - total ionization; 2 - $He^+$ heavy nuclei; 3 - heavy nuclei; 4 - radiation belt near the Earth; Plot b - along the ordinate - microrep/24 hours, along the abscissa - altitude, km; 1 - ionization peaks (Schaefer, 1952).

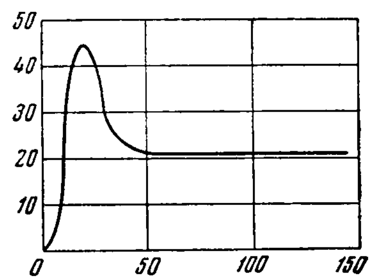

Figure 2. Intensity of Cosmic Radiation in the Atmosphere at Various Altitudes above Sea Level. Along the abscissa - altitude, km; along the ordinate - number of pulses in a single counter per second (Dobrotin N.A.)

The intensity of ionization at an altitude of 22-23 km, which corresponds to 15 mrep/24 hours, proves that the effect of the radiation is very near to the one assumed at present as the maximum permissible dose for continuous (chronic) irradiation, equalling 100 mrep or 0.1 rem per week (Recommendations ..., 1959). [Translator's note: The Soviet definition of a "rem" differs slightly from the standard U.S. definition. According to the Soviet definition, 1 rem is that quantity (or dose) of any type of ionizing radiation ($\alpha, \gamma$, neutron, X-ray etc.) which causes "biological" (physiological) damage equivalent to the effect of 1 roentgen.] Under certain conditions (geomagnetic latitude, secondary processes caused by cosmic radiation, periods of solar activity, radiation belt etc.), this dose can be considerably exceeded.

The magnetic field of the Earth has a great influence on the intensity and qualitative composition of the cosmic rays which impinge on the upper layers of the atmosphere. This proves that the primary cosmic radiation consists of a flux of charged particles. The equatorial region can be penetrated only by particles of very high energies. The average energy of the cosmic-ray particles changes with the geomagnetic latitude: from 1 Bev (high latitudes) to 4 Bev (low latitudes) - per nucleon. The minimum energy of the proton is: 0.35 Bev at a latitude of $55^{\circ}$, 0.5 Bev at $51^{\circ}$ and 3.5 Bev at $30^{\circ}$ (Tobias, 1956). Protons with energies of $10^{10}$ ev may reach any part of the Earth's surface in the vertical direction (Vernov, Chudakov, 1960).

At the ground surface the difference in the radiation intensity at various geomagnetic latitudes is relatively small, it is appreciably higher in the stratosphere and in the upper layers of the atmosphere the radiation intensity fluctuates between wide limits. Above the equator the radiation intensity is one-third of that observed at latitudes above $55^{\circ}$ and three times higher than in areas of medium latitude. These differences are explained by the dependence of the intensity of the cosmic radiation on the geomagnetic latitude, which is basically due to low-energy cosmic-ray particles, the influence of which hardly extends to the lower layers of the atmosphere. Figure 3 shows a few curves of the dependence of the ionization on altitude for various geomagnetic latitudes.

Thus, the intensity of cosmic rays impinging on the upper layers of the atmosphere in the polar regions will be higher than the above given average values and a distinguishing feature of their qualitative composition is their relatively higher content of low-energy particles. It will be seen later that the low-energy particles probably have the highest biological effect.

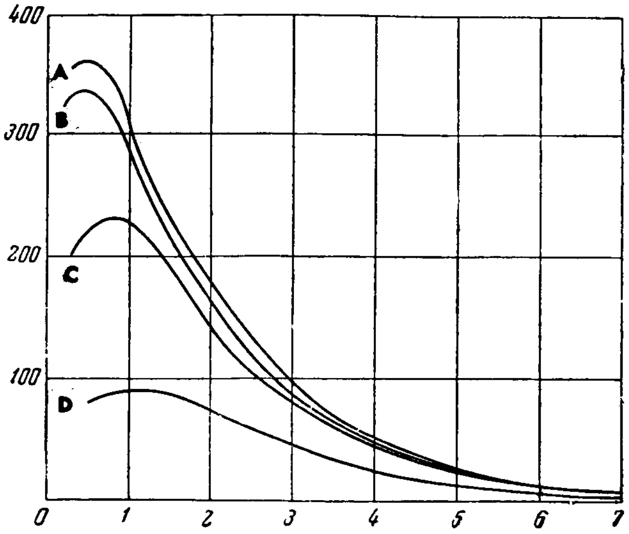


Figure 3. Intensity of Ionization as a Function of Altitude for Various Geographical Latitudes. Along the ordinate - pair of ions/cm$^2$/sec; along the abscissa - pressure in water col.m. A - 60° northern latitude, B - 51° northern latitude, C - 36° northern latitude, D - 3° northern latitude (Shepherd, 1953)

At polar latitudes exceeding 55-60°, no further increase in the intensity of cosmic radiation could be observed; throughout the entire equatorial zone (from 0 to 15° northern and southern latitudes) their intensity remained the same. Furthermore, the ratios of the nuclei of various elements are almost equal throughout all latitudes, although the atomic weight and the energies of these nuclei differ (Shepherd, 1953; Tobias, 1956; Nervichnoye kosmicheskoye izlucheniye (Primary Cosmic Radiation), 1956; Neher, 1958 and others).

As was mentioned above, due to secondary processes, cosmic rays produce maximum ionization at an altitude of 22-23 km (Figure 1), i.e. after penetrating a layer of air of a thickness of 35 g/cm$^2$. The rate of energy loss by cosmic rays at this altitude increases by almost two times (compared with the upper boundary of the atmosphere). If shields of a denser material (for instance, metal) were to be placed into the upper layers of the atmosphere instead of the rarefied air, then, at a certain thickness, the intensity of the ionizing effect of the radiation

penetrating through the shield could increase by more than four times - "transient effect". This is one of the difficulties of developing means of protection for shielding living beings from the effects of cosmic rays (Schaefer, 1952).

Experimental data obtained by means of rockets and balloons have shown that the intensity of cosmic rays is subjected to continuous fluctuations (the so-called intensity variations), which depend on numerous factors, and particularly that they are correlated with the cycles of solar activity: diurnal, 27-day, annual, 11-year cycles, etc., (Dobrotin, 1954; Vernov and others, 1957, 1959, 1960; Kurnosova and others, Neher, 1958; Schaefer, 1958a and others).

It can be seen from Figure 4 that in a year with a high solar activity (1937) the level of radiation was lower, while in a year with a low activity it was higher. In addition, in years with a high solar activity, the fluctuations (variations) of the intensity of the cosmic rays are more pronounced. The indicated changes in the spectra of the primary particles and the appearance of particles with low energies in years with minimum solar activity have so far not been fully explained. There is reason to believe that, beyond the limits of the solar system, the lower threshold of the energy of the cosmic-ray particles is lower than within the limits of this system (Shepherd, 1957).

The Sun itself is a powerful source of relatively low-energy electromagnetic and corpuscular radiation. Only two small sections of the

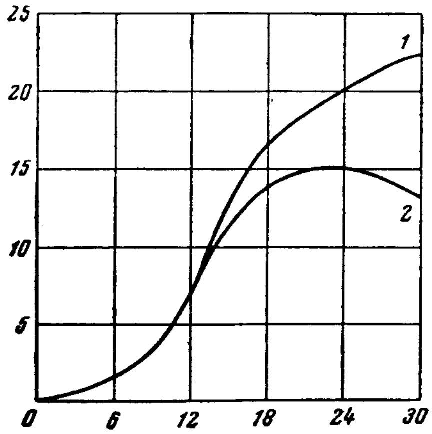


Figure 4. Ionization at Various Altitudes During a Year With a High Solar Activity (1937) and During a Year With Low Solar Activity (1954). Along the ordinate - altitude, km; along the abscissa - total ionization, millirad/24 hours. 1 - 1954; 2 - 1937 (Shepherd, 1954)

wide spectrum of solar radiation penetrate to the surface of the Earth: the visible, infrared light and radio waves of 1 cm to 20 m (Tobias, 1959). The average energy of ionizing radiation of the Sun is incomparably lower than the energy of particles of the primary cosmic radiation (by $10^5$ times), it has, however, a considerably higher intensity (particle flux density). Solar radiation has a great influence on the state of the ionosphere and is the cause of magnetic storms and aurorae.

Solar flares are accompanied by a sharp rise in radiation intensity. During, and for several hours after, flares, the radiation dose in the upper layers of the atmosphere may equal several rads per hour (for instance, February 23, 1956) and may even be higher. During flares, the energy spectrum of the radiation changes considerably; the energy of solar $\gamma$-rays may reach 3-5 Mev (possibly of secondary origin); protons appear with energies of several hundred Mev, and also probably a certain quantity of $\alpha$-particles and multicharged nuclei. Figure 5 shows examples of increase in the intensity and changes in the energy spectrum during flares.

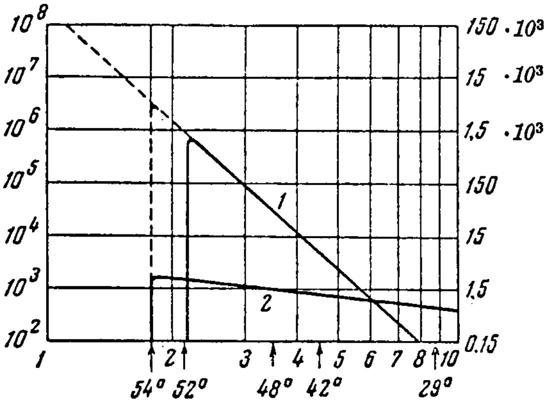


Figure 5. Changes in the Intensity and the Energy Spectrum of Protons During Solar Flares. Left ordinate - protons/$m^2$sec·sterad; abscissa - energy $10^9$ ev for various latitudes. Right ordinate - integral dose during a flare milliard-rad: 1 - flare; 2 - norm (Schaefer and Golden, 1960)

Solar flares may present a serious radiation danger to space travellers. Unfortunately, with the present state of knowledge, we cannot always predict their appearance. Optical observations permit only to distinguish the beginning of such flares. It is true that the pronounced optical picture of a flare does not always correspond to an increase in the intensity of ionizing radiation. The so-called "solar radio noise" is more correlated with an increase in the intensity of cosmic rays.

Powerful flares are of rarer occurrence, while relatively weak flares are more frequent. Therefore, counting the number of flares during a certain time is of a relative nature (is of a conditional character). For instance, during the IGY one intensive flare per month on the average was recorded.

Only slight changes in the level of radiation at the ground surface (not exceeding 20%) will correspond to the sharp fluctuations of the radiation intensity in the upper layers of the atmosphere. In the range of high magnetic latitudes, these are more pronounced. These fluctuations are due predominantly to the low-energy components. However, the measurements carried out by means of the second Soviet space rocket enabled the detection of cases of a larger increase (by ten times) in the intensity of flow of nuclei with charges exceeding 15, which were correlated with the radio-radiation of the Sun (Trudy Konferentsii po issledovaniyu Solitsa (Transactions of the Conference on Investigating the Sun), 1957; Krasovskiy and others, 1958; Stantsii v kosmose (Space Stations), 1960; Charakhch'yan and others, 1961; Gorchakov, Bazplevskaya, 1961; Ellison, 1959; Massey, 1959; Burnight, 1952; Schaefer, 1958a, 1958b; Tobias, 1959 and others).

In zones of so-called atmospheric "showers", the energy release of the cosmic radiation increases sharply compared to the average level (by about $10^6$ times). However, due to the extremely short duration of the "shower" (millionths of a second) and the low statistical probability of its repetition at a given point, the total dose of radiation of any object during a specified period of time will not change appreciably.

The recently discovered powerful radiation belts surrounding the Earth increase appreciably the radiation danger of space flights. The discovery of radiation belts leads to the assumption that from the radiation point of view many unexpected things will be encountered in space.

Conditions exist which lead to the assumption that in space large accumulations of charged particles move in the form of "plasma", which approach the Earth near its poles. Passage of a spacecraft through such zones may present considerable danger (Schaefer, 1958 and others). During solar flares accumulations of ionized plasma in the form of "magnetic clouds" may separate from the Sun and approach the Earth, causing magnetic storms and sharp changes of the radiational field in the neighborhood of the Earth and within the limits of the solar system (Tobias, 1959).

Thus, from the radiation point of view space is nonuniform. It is necessary to distinguish between the so-called free cosmic space and the space directly near celestial bodies where, under the influence of the magnetic field, and the reflection or shielding of charged particles by

the celestial body, the level and properties of the radiation may change greatly (Strughold, 1959; Van Allen, 1960). The results obtained by means of Soviet space rockets have shown that there is no zone of increased cosmic ray intensity in the neighborhood of the Moon; this indicates absence of a magnetic field near the Moon (Stantsii v kosmose (Space Stations), 1960; Kurnosova and others, 1960; Vernov, Chudakov, 1960; Vernov, Chudakov and others, 1960; Van Allen, 1960).

It is necessary to study carefully the complex pattern of the radiation field in the neighborhood of the Earth and further away from it in space. It is also necessary to bear in mind that the configuration of the radiation field and its intensity also change with time.

Radiation Belts Surrounding the Earth

The outstanding successes achieved in recent years in studying space, expressed in terms of launching artificial satellites and space rockets, have permitted discovery of new and greater possibilities for studying the cosmic rays and their biological effect. High altitude rockets have allowed measurement of the radiation level in the upper layers of the atmosphere for no more than a few minutes, while an artificial satellite permits obtaining information on the intensity of radiation along its orbit at various geographical latitudes and longitudes over long periods. Space rockets extend still further our potentialities. They permit the study of the radiation field in depth at any distance from the Earth (Vernov, Chudakov, 1960; Vernov and others, 1960; Kurnosova and others, 1960). Indeed, from the time of launching of the first artificial Earth satellite (October 4, 1957) our knowledge of cosmic rays has been greatly enriched.

Investigations carried out by artificial satellites have revealed the existence at an altitude of the order of thousands of kilometers of an unexpectedly high level of radiation, exceeding by thousands of times the radiation anticipated in space (Vernov and others, 1957, 1957a, 1958, 1958a; Kurnosova and others, 1958; Van Allen et al., 1958, 1958a, and others). During its orbit, the recorded level of radiation in the satellite changes within a wide range. The intensity of radiation changes both with the height above the Earth and with the geographical coordinates. Furthermore, an increase of many times has been observed in the level as well as an increase in the range of fluctuations of cosmic radiations, which is obviously associated with the state of the solar activity, the state of the magnetic field of the Earth and phenomena occurring in the ionosphere.

Artificial satellites and space rockets have enabled detection of radiation belts surrounding the Earth which are distributed parallel to the equator and to lower latitudes. Two belts (zones) of high radiation

intensity are separated from each other by a space with a relatively low radiation intensity.

The internal belt, nearer to the Earth, extends from an altitude of about 500-600 km (above the equator) to 5-10 thousand km and is located between 35° northern and 35° southern latitudes. The maximum radiation intensity in this belt is at an altitude of about 3400 km. The outer belt, more distant from the Earth, is wider and extends between 60-65° northern and southern latitudes from an altitude of 14-15 thousand km to 50-55 thousand km above the Earth's surface. According to measurements made with the first space rocket, the maximum radiation intensity in the outer belt was recorded at an altitude of 26,000-27,000 km and with the second space rocket at an altitude of 17,000 km (Kurnosova and others, 1960; Vernov, Chudakov, 1960). From an altitude of 500-600 km onwards the radiation intensity doubles for every 100 km altitude until a maximum is reached inside the internal belt, then it starts to decrease (Tobias, 1959).

Beyond the limits of the radiation belts the intensity of the cosmic-ray flux is about 2 particles per 1 $cm^2$/sec, as was assumed earlier, and the ionizing ability is only 2.5 times the minimal; in the zones of the radiation belts near the Earth these equalled tens of thousands of particles (the recording was carried out by counters protected with certain shields). Figure 6 shows the trajectory of one of the lunar rockets [Translator's note: "Pioneer IV"] and gives the recorded number of particles during the flight, while Figure 7 illustrates diagrammatically the configuration of the zones of high radiation intensity (in section). The isodoselines indicate the drop in intensity of the radiation from the center of the zone towards its periphery. The radiation intensity in the outer belt decreases slowly with increasing distance from the Earth, gradually approaching the background radiation in the free cosmic space (60-70 thousand km). Figure 8 shows the total intensity (energy) of cosmic radiation in electronvolts impinging on 1 $cm^2$/1 sec recorded as a function of the distance from the Earth. The maximum energy amounted to $10^{11}$-$10^{12}$ and more electronvolts (Stantsii v kosmose (Space Stations), 1960; Vernov, Chudakov, 1960; Van Allen and Frank, 1959 and others).

The high-energy protons (tens and hundreds of Mev) form an important part of the radiations of the inner belt. In addition, in the inner belt electrons are present (particularly at the edges of the belt), the energy of which does not exceed 1 Mev. Table 1 gives data published by Van Allen and Frank (1959) on the composition and intensity of the radiation

in the inner belt as measured by means of the lunar rocket "Pioneer IV".

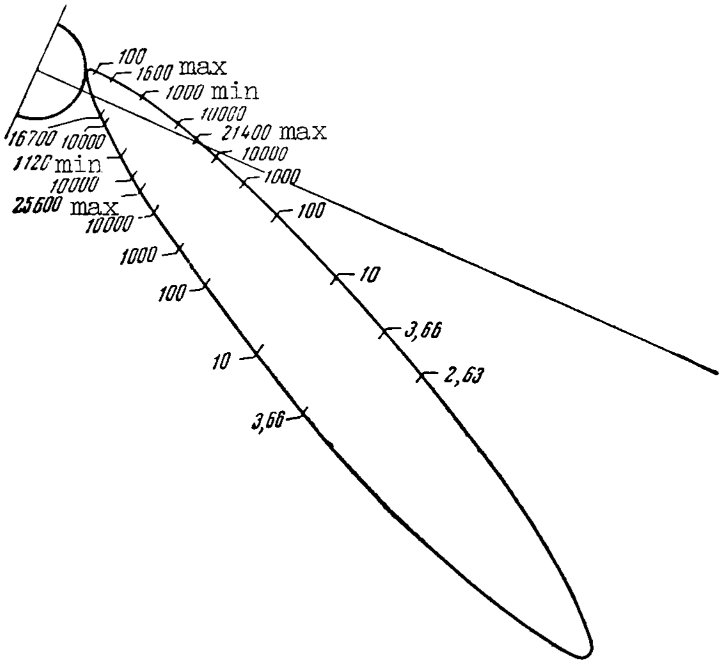


Figure 6. Trajectory of One of the Lunar Rockets ("Pioneer IV"). The figures indicate the number of particles recorded by the counter (Van Allen)

The penetration ability of the radiations of the inner belt is very high. If the shielding is increased from 140 mg/cm$^2$ to 1 g/cm$^2$, the radiation intensity decreases sharply; however, if the shielding is further increased the decrease will be very slow (Van Allen, 1960; Vernov, Chudakov and others, 1960). Of 3000 particles/cm$^2$·sec which penetrate through a protection of 1 g/cm$^2$, 60% will penetrate through 2.5 g/cm$^2$ (Van Allen, 1960). Under a protection of 1 mg/cm$^2$, fluxes with energies up to 100 erg/cm$^2$·sec·sterad (Vernov, Chudakov and others, 1960) were discovered. The radiation under a protection of 1 g/cm$^2$ in this belt is, according to Van Allen (1960), about 10 r/hour. S.N. Vernov and A.Ye. Chudakov (1960) have shown that probably almost 1/1000 of the energy of the particles is spent on producing radioactive isotopes in the surrounding (irradiated) medium.

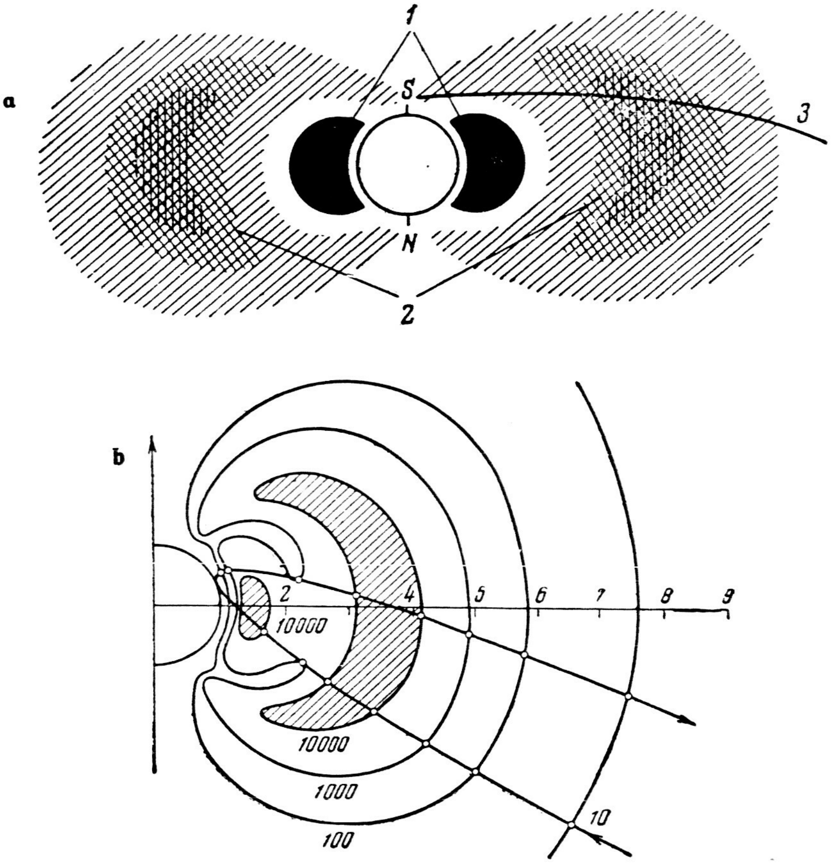


Figure 7. Configuration of Zones of Increased Radiation Surrounding the Earth. a - sketch of the distribution of the inner and outer zones: 1 - internal belts; 2 - external belts; 3 - trajectory of the first space rocket; b - on the horizontal line are plotted distances in Earth radii. The concentric lines circumscribe zones of different radiation intensities (the figures indicate the number of particles recorded by the counter) ("Pravda"; Van Allen)

The particles of the outer belt consist basically of electrons with a much lower energy (10-50-100 kev) and penetrating ability. Particles with a penetration capability of several $g/cm^2$ are almost absent in the outer belt. Table 2 shows the data of S.N. Vernov, A.Ye. Chudakov and others (1959, 1960) and Van Allen (1959) on the composition of the radiations in the outer belt.

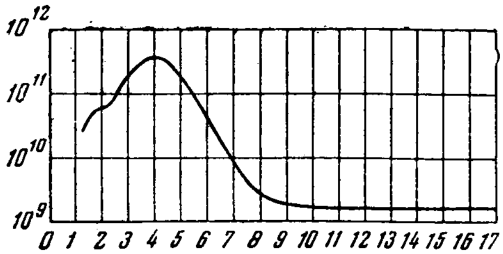


Figure 8. Plot of the Changes in Radiation Intensity as a Function of Distance from the Earth. Along the ordinate - ev/1 cm/sec; along the abscissa - Earth radii ("Pravda")

Table 1. Inner Belt (Altitude 3600 km above the Earth)

| Particles | Energy | Intensity (particles per $cm^2 sec \cdot sterad$) |
|---|---|---|
| Electrons | > 20 kev | $2 \cdot 10^9$ |
| | > 600 kev | $1 \cdot 10^7$ |
| Protons | > 40 Mev | $2 \cdot 10^4$ |

Thus, in the outer belt all the electrons moving in all directions, including electrons with a minimal energy, amount to about $10^{10}$ per $cm^2$/sec. Such a flux of electrons can produce a surface irradiation dose of the order of $10^6$ r/hour or up to 40,000 rad per second. Under a protection of 1 g/$cm^2$, the radiation dose in the center of the outer belt is probably near to 50 r (Vernov, Chudakov, 1960; Stantsii v kosmose (Space Stations), 1960; Van Allen, 1960; Tobias, 1959).

Protection from most of the electrons of the outer belt is relatively easy. In the inner belt, 4 g/$cm^2$ lead will only slightly reduce the radiation dose, while in the outer belt such a shield would reduce radiation by a factor of 50 (Van Allen, 1960).

Table 2. Outer Belt

| Particles | Energy | Intensity, particles per $cm^2/sec \cdot sterad$ | Source |
|---|---|---|---|
| Electrons | 20-50 Mev | $10^{10}$ | S.N. Vernov, A.Ye. Chudakov and others (recording was by means of scintillation counters covered with a layer of Al 1 $g/cm^2$) |
| " | 100 kev- 1 Mev | Up to $10^5$ | |
| " | > 2 Mev | $5 \cdot 10^5$ | |
| " | > 5 Mev | 1 | |
| Electrons | > 20 kev | $10^{11}$ | Van Allen and Frank |
| " | > 200 kev | $10^{11}$ | |
| Protons | > 2.5 Mev | $10^6$ | |
| " | > 60 Mev | $10^2$ | |

Solar flares and changes in solar activity cause a sharp change in the intensity of radiations both in the free outer space as well as in the belts surrounding the Earth. The outer belt is subjected to fluctuations of the radiation level, which are associated with solar activity and other factors, within very wide limits (during flares the intensity increases by thousands of times), the inner belt shows relatively few fluctuations in intensity - up to 15% (Vernov, Chudakov, 1960; Stantsii v kosmose (Space Stations), 1960; Van Allen, 1960; Langham, 1959, and others).

The space between the inner and outer belts (the so-called wedge) is located at 10-14 thousand km from the surface of the Earth. Within this "wedge" a relatively low radiation dose has been recorded: under a protective shield of 1 $g/cm^2$ the radiation dose equalled about 0.4 r/hour. A protection of 4 $g/cm^2$ reduces the dose by a factor of four. The flux of electrons with energies in excess of 100 kev and of protons with energies above $10^8$ ev is about 1000 times lower in the range of the "wedge" than in the inner belt (Vernov, Chudakov, 1960). As regards a number of its characteristics, the "wedge" assumes an intermediate position between the inner and outer belts: the penetrating ability of the radiation is here lower than in the inner and higher than in the outer belts, while the fluctuations in the radiation intensity are more pronounced in the inner and less pronounced than in the outer belts (Van Allen, 1960).

In the radiation belts near the Earth, high-energy photons were also recorded, which indicates the existence of a very powerful bremsstrahlung. This radiation is obviously due to the interaction of electrons (100 kev—1 Mev) with the shell of the counter and may have a considerable penetrating ability and energy in excess of 100-400 kev and even 1 Mev (Vernov, Chudakov and others, 1960; Stantsii v kosmose (Space Stations), 1960). The formation of X-ray radiation is associated with deceleration in the shell of the satellite capsule and of the spacecraft of electrons with energies of 2-3 Mev, and these present considerable danger to space travellers.

The detected density of charged particles and the total radiation energy passing through per unit of surface, per unit of time, in the radiation belts surrounding the Earth (Figures 6, 7) lead to the assumption that in zones of maximum radiation intensity the ionizing effect inside the spacecraft reaches magnitudes equivalent to 5-10 (if caused by electrons) and even 50-100 (for protons) r/hour (Van Allen and Frank, 1959).

In the formation of radiation belts near the Earth the magnetic field of the Earth plays a decisive role. The charged particles are retained near the Earth by the magnetic field, the dipole character of which leads to the formation of a "trap". Various views exist relating to the origin of the charged particles inside the belts.

It is possible that in the mechanism of formation of the outer belt, particles of solar origin (electrons), which are captured and accelerated by the magnetic field of the Earth, play a major role. The origin of the inner belt can be visualized as follows: under the influence of primary cosmic rays, the Earth's atmosphere emits neutrons which when flying beyond the Earth decompose into electrons and protons which make up the belt (Vernov S.N. and Lebedinskiy A.V., 1958; Vernov, Chudakov, 1960; Shklovskiy I.S. and Krasovskiy V.I., 1959; Van Allen, 1960; Van Allen et al., 1959).

There is also a hypothesis according to which the particles of the inner belt form artificially as a result of high-altitude explosions carried out in recent years in the U.S.A. In the "Argus" experiment, a small atomic device was exploded between the two zones. This experimental explosion caused an increase in the radiation intensity in the inner radiation belt which is nearer to the Earth. The results obtained in the experiment were discussed in a special symposium. These enabled certain assumptions to be made on the total number of particles in the zones as well as on the role of atomic-bomb explosions on the origin of radiation belts surrounding the Earth (Shklovskiy I.S. and Krasovskiy V.I., 1959; Experiment "Argus", 1960; Van Allen, 1960; Tobias, 1959, and others).

In the near-Earth belts, the charged particles obviously move along close trajectories, "twisting themselves" onto the magnetic lines of force. The particles move parallel to the surface of the Earth and the movement probably continues for a very long period.

The radiation belts surrounding the Earth are located symmetrically relative to the geomagnetic equator, which is tilted at a certain angle (11-15°) relative to the geographic equator. Due to the displacement of the magnetic dipole, relative to the center of the Earth, by 360 km towards the eastern hemisphere, the radiation belts are shifted somewhat relative to the globe: in the eastern hemisphere the inner belt begins at an altitude of 1500 km, in the western hemisphere it begins at an altitude of 500 km from the ground surface (Stantsii v kosmose (Space Stations), 1960; Vernov, Chudakov, 1960; Trudy Mezhdunarodnoy konferentsii po kosmicheskim lucham (Transactions of the International Conference on Cosmic Rays), 1960; Savenko, Shavrin and others, 1961; Gorchakov, 1961; Vernov, Savenko, Shavrin and others, 1961; Ginzburg, Kurnosova and others, 1961).

It can be seen from the sectional diagram in Figure 6 [Translator's note: Figure 7] that the outer belt is in the shape of a half-moon and comes nearest to the Earth at 60-65° (55-70°) northern and southern latitudes, dropping down to an altitude of 270-300 km (Stantsii v kosmose (Space Stations), 1960). Here, the charged particles of the belt penetrate into the atmosphere of the Earth and probably play a major role in the formation of the aurorae which are most frequently observed in these latitudes. According to other data (Gorchakov, 1961), aurorae occur as a result of direct penetration into the atmosphere of particles emanating from the Sun and the boundary of the outer radiation belt is at lower latitudes (55-60°) than the zone of maximum aurorae (70°).

On penetrating into the atmosphere and interacting with it, the electrons form X-ray radiations which at an altitude of about 100 km bring about ionization and light emission in the denser layers of the atmosphere. During this process, longer wave electromagnetic oscillations may be generated which influence the temperature of the atmosphere (Ellison, 1959 and others). It is assumed that in the zone of aurorae the energy of X-rays is 10-100 kev. Electron fluxes with energies of 10 -100 kev may yield $10^8$ electrons per 1 $cm^2 \cdot sec$ with a total dose of 500 mrad/hour or 10 rad/24 hours. At an altitude of 30 km (this point is separated from the zone of aurorae by a layer of air of 8 $g/cm^2$) a dose of only 5 mrad per 24 hours was recorded, which indicates that this radiation has a low penetration power and that methods of protection against it are available (Schaefer, 1958; Van Allen, 1958, 1960, and others).

Penetration of electrons from the outer belt into the atmosphere depends to a large extent on the state of solar activity: if this increases, the steady-state conditions in the magnetic field are upset, and the electrons can "fight their way out" from the "magnetic trap" to the Earth (Stantsii v kosmose (Space Stations), 1960). From the inner belt the particles become dissipated, mainly by the atoms of the atmosphere of the Earth. This determines the lower limit of the radiation belt (600 km), where the barometric pressure begins to increase more noticeably (Van Allen, 1960; Tobias, 1959; Ginzburg, Kurnosova and others, 1961; Vernov, Savenko, Shavrin and others, 1961).

Recently, in the experiment with the second spaceship, the high radiation intensity which is characteristic for the belts surrounding the Earth was also detected in some regions at a considerably lower altitude (of the order of 300 km). With a high degree of stability this was detected above the southern part of the Atlantic Ocean (on one of the orbits of the spaceship similar phenomena were observed also in the northern hemisphere). Such "flows" of particles from the near-Earth belt are explained by the strong anomalies in the magnetic field near the Earth (Kurnosova and others, 1961; Ginzburg, Kurnosova and others, 1961; Vernov, Savenko, Shavrin and others, 1961). This shows again that for materializing future space flights it is necessary to study in great detail the shape and all the features of the radiation field beyond the limits of the atmosphere.

The composition of the charged particles of the primary cosmic rays and their energy spectrum have been relatively well studied but very little is known on the biological effect of the heavy particles which have very high energies. As regards the near-Earth radiation belts, the opposite was found to be valid: the biological effects of radiation can be relatively easily evaluated with the present state of knowledge but much is still unknown as regards its physical characteristics.

In the distant future, spaceships will probably travel with a velocity approaching the speed of light (relativistic speed). At such velocities the number of charged particles penetrating into the spacecraft per unit of time and the total dose of radiation would increase catastrophically. The dose of radiation on the surface of the spacecraft could reach 20,000,000 r/hour, and the energy of the particles relative to the craft would also increase in this case. Under such conditions the problems of protection become extremely important (Van Allen, 1960).

## Interaction Between Cosmic Radiation and the Medium, Density and Pattern of Ionization

After penetrating into the atmosphere or into any medium in their path, cosmic rays are subjected to a number of transformations, the final result of which is ionization and destruction of the nuclei of the

medium. For judging the biological effect of cosmic radiation, it is necessary to have available detailed information on the stages of the energy transformation in the primary particles and the process of formation of secondary radiation.

After penetrating any type of medium, the primary cosmic-ray particles may lose energy as a result of two basic processes: 1) gradually by ionization and excitation of atoms of matter through which they pass; 2) by collisions with atomic nuclei of the matter.

In the latter case various nuclear reactions take place. The effect of disintegration of the nucleus and the formation of fragments which scatter in various directions is referred to as the formation of "stars" (this is what these phenomena look like on nuclear photo-emulsions).

The energy of the primary particles becomes distributed between the fragments and the nascent secondary particles. Formation of the secondary particles is a complex multistage process. On the average in the "stars" there are 50% protons, 30% $\alpha$-particles, 10% deutons, 3-4% tritons and heavy particles form only an insignificant percentage. Furthermore, mesons are formed which carry away a considerable fraction of the energy (Dobrotin, 1954 and others).

Most of the primary particles lose a known part of their energy as a result of ionization, and then on colliding with the nucleus they transmit a considerable part of the energy to secondary particles. The probability of collision of a primary particle and formation of a "star" is determined by the energies of the particles and the effective cross-section of the interaction of particles in the nuclei of the substance through which it passes (the nuclei of the "target"). Substances in which the ratio of the effective cross-section of the nucleus to its weight is larger, yield the highest number of collisions of primary particles per unit of weight of the substance. These properties are possessed by light substances (hydrogen, water, etc.). Table 3 gives the values of the mean free path of particles (reduction of the particle flow by 37%) in air and in iron.

Table 3.

| Primary particles | Mean Free Path, $g/cm^2$ | |
|---|---|---|
| | Air | Iron |
| H | 85 | 120 |
| He | 51 | 90 |
| Na | 29 | 60 |
| Mg | 23 | 50 |
| Fe | 15 | 37 |

Some particles terminate their free travel without collision. This applies primarily to low-energy particles basically below 100 Mev per nucleon. The higher the energy of the primary particle which approaches the boundaries of the atmosphere, the higher will be its probability of collision (Figure 9).

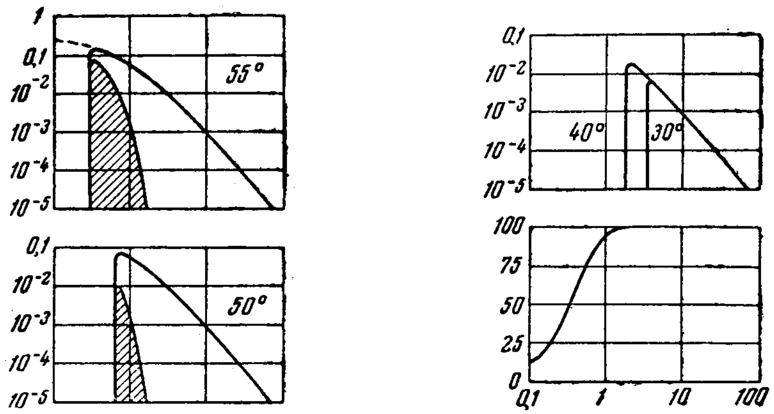


Figure 9. Energy Spectrum of Primary Cosmic Particles (for the Group C-N-O) at the Upper Edge of the Atmosphere at Various Latitudes and the Probability of Formation of Ionization Peaks and "Stars". Along the abscissa (for all the plots) - kinetic energy, $10^9$ ev; along the ordinate - particles/sec•sterad, $m^2$ (for the top three plots); percentage of particles forming "stars" (bottom plot). The shaded sections indicate the number of ionization peaks (Schaefer, 1954).

At the end of the path of a particle, the rate of energy loss and, correspondingly, the ionization density increase sharply. In this part of the path a pronounced ionization peak is observed (Figure 10), which terminates with the last section of travel of the particle characterized by a drop in ionization and a velocity of the particle caused by capture of electron particles and a reduction of its effective charge. Study of the traces of particles in photo-emulsions shows a widening of the trace with a final thindown phenomenon. Obviously, due to the very high ionization density, referred to as ionization peak, this section will have the greatest biological effect. The ionization peak and the thindown phenomenon of the trace are characteristic for heavy particles.

Probably formation of ionization peaks are to be anticipated only above high latitudes (over 50°) where primary particles of a relatively

low energy of up to 1 Bev can penetrate (Schaefer, 1952, 1954, 1958b, and others). At the upper boundary of the atmosphere, a considerable part of the C-N-O nuclei passes right through the human body without causing any ionization peaks. The maximum number of ionization peaks will occur if the body is located at a certain depth inside the atmosphere where the average energy of the nuclei is lower. Since the mean free path of heavier nuclei (for instance Fe) is lower, for these the ratio will be somewhat different (Figures 9, 11).

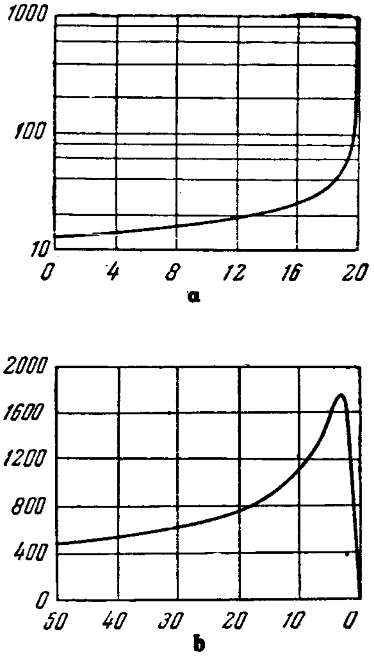


Figure 10. Ionization Caused by Protons in the Tissue. Along the ordinate - ion pairs per micron of the path; along the abscissa - depth of penetration into the tissue, cm (plot a) and distance to the final point of travel, $\mu$ (plot b) (Schaefer, 1960)

The ionization density caused by the passage of a charged particle through any medium is proportional to the density of the material of this medium, and to the square of the particle charge, and shows a complex dependence on the speed of movement. The ionization density is lowest at a speed equalling 0.97 c (c - speed of light). If the velocity of the particle decreases, the ionization density increases rapidly. In the range of moderate velocities, the ionization density is approximately inversely proportional to the square of the velocity. However, at velocities at which the primary particles travel, this dependence has little effect.

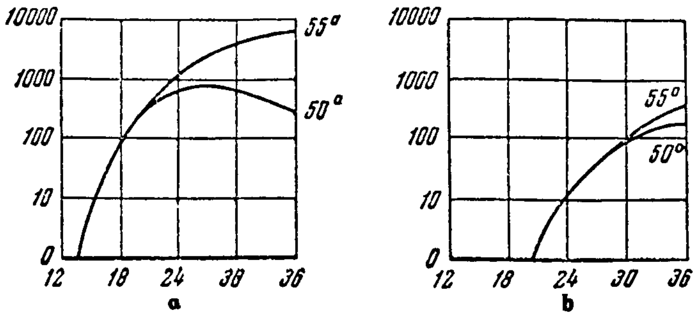


Figure 11. Number of Ionization Peaks Generated in the Human Body by Heavy Nuclei, as a Function of Altitude and Geomagnetic Latitude. Along the abscissa - altitude, km: along the ordinate - number of ionization peaks per hour. Plot a - C-N-O nuclei; Plot b - Fe (z = 26) (Schaefer, 1954)

In forming one ion pair, 32-35 ev of energy is expended. Thereby, simultaneously with the formation of a pair of ions, electrons of 2-5 molecules are excited. The length of the free path of a particle in a given medium is directly proportional to the kinetic energy of the particle and inversely proportional to the square of its charge. The total ionization caused by the particle throughout its trajectory is fully determined by the energy of the particle; for instance, a proton with an energy of $3 \cdot 10^9$ ev (the average energy of cosmic-ray protons) may form 100,000,000 pairs of ions (if it does not collide with the nucleus of a medium).

After collision of the particles with a nucleus and formation of a "star", ionization becomes more diffuse and scattered in the mass of the medium (Shepherd, 1953; Tobias, 1956, and others).

The curve of the dependence of the specific ionization on the kinetic energy of a proton is plotted in Figure 12. For air the minimum ionization caused by a proton amounts to 28,000 ion pairs per unit of the trajectory expressed in g/cm$^2$; for the tissue of the human body it amounts to 30,000 or three pairs of ions per 1$\mu$ of the trajectory. To calculate the specific ionization for multi-charged particles, the ordinate of the graph of Figure 12 has to be multiplied by the square of the particle charge.

Protons (with energies of 0.56-13 Bev) and electrons produce a minimum ionization density - three pairs of ions, about 12 $\alpha$-particles (300 Mev - 8 Bev), 150 to 1000 C-N-O nuclei, 2000-4000 ion pairs per 1$\mu$ of trajectory in biological tissues. With the slowing down of the nuclear particles, the rate of energy loss and the corresponding density of generated ionization increases sharply. Thereby, protons may form about 1600, $\alpha$-particles 5500, heavy nuclei from 35,000 (C-N-O) to 100,000 (Fe - on lowering the energy to 5 Mev) ion pairs per 1$\mu$. Thus, an iron

nucleus can form up to 2,000,000 pairs of ions in one 10$\mu$ diameter cell of the organism (Shepherd, 1953).

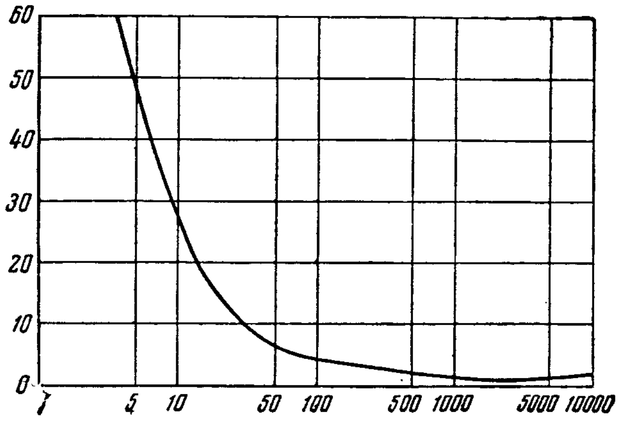


Figure 12. Curve of the Dependence of the Ionization Density Produced by the Proton on its Kinetic Energy. Along the abscissa - energy, Mev; along the ordinate - $j/j_{min}$: j - specific ionization, $j_{min}$ - minimum ionization at the velocity 0.97 c (Shepherd, 1953)

The soft and hard components of cosmic radiation which reach the Earth's surface ($\mu\pm$ mesons, $\gamma$-quanta, electrons, positrons, as well as superfast protons and neutrons) produce on the average a minimum ionization (70-80 pairs of ions per 1 cm of the trajectory in air or three pairs per 1$\mu$ in tissue). The protons, neutrons, deuterons, tritons and fragments of nuclei with energies of 10-15 Mev cause very intensive ionization.

The ionization caused by the final section of the mean free path of heavy particles (ionization peak) is so intensive that its relative importance from the point of view of the total ionization is very great. Therefore, Schaefer (1952, 1958), Tobias (1956) and others distinguish: total ionization; ionization caused by ionized peaks of heavy particles; ionization produced during collisions of primary particles with nuclei of the medium (stars). The relations between these types of ionization differ at different altitudes and different geomagnetic latitudes.

Although only 1% of the primary particles are heavy nuclei ($Z > 2$), their energies amount to 40-50% of the total energy of the cosmic radiation (Tobias, 1956). The ionization from a single iron nucleus is 676 times as much as that from a proton. According to the data of Schaefer (1958), the total ionization effect caused by the iron nuclei in cosmic radiation and expressed in physical roentgen equivalents (reps) is only

a quarter of the total ionization produced by protons. Taking into consideration the assumed biological equivalent of the iron nuclei, their effect will be four times as high as the effect caused by the protons (Table 4).

Table 4. Composition of the Primary Cosmic Radiation beyond the Earth's Atmosphere for 55° with the Geomagnetic Latitude (Schaefer, 1958b)

| Type of nuclei | | H-protons | He-α-particles | C-N-O | Mg | Ca | Fe | Total dose from all components |
|---|---|---|---|---|---|---|---|---|
| Intensity, number of particles passing per hour through the cross-section of a sphere with an area of 1 $cm^2$ | | 4460 | 633 | 32 | 8.4 | 2.9 | 1.4 | |
| Ionization of the tissue (milliroentgen per 24 hours) | | 4 | 2.3 | 1.4 | 1.1 | 1.1 | 0.9 | 10.7 |
| Speed of loss of energy, ion pairs per micron of travel in the tissue | Min | 6 | 24 | 300 | 865 | 2400 | 4050 | |
| | Max | 1650 | 7200 | 35,200 | 50,900 | 73,400 | 100,000 | |
| Effect on the cell (the average ionization occuring in a cell of 10μ dia. during passage through it of one particle with an atomic number between 7 and 26 is taken as the unit) | Min | 0.07 | 0.24 | 0.36 | 1.0 | 2.85 | 4.8 | |
| | Max | 20 | 85 | 420 | 620 | 870 | 1200 | |
| Biological dose, mrem/24 hours | | 4 | 2.3 | 3.6 | 7.0 | 14.0 | 16.0 | 46.9 |

Since the biological effects are determined to a great extent by the ionization density or by the velocity of the energy loss of the particles, the concept of the mean specific ionization or the mean specific loss of energy by all the particles at a given altitude is introduced.

Numerically this quantity can be obtained from the ratio of the total ionization to the number of particles recorded by the counter at this altitude. The curve of the dependence of the specific loss of energy on altitude above ground, given in Figures 1 and 13 (curve B), illustrates particularly clearly the manifestation of the transient effect and of the maximum ionization at 22-23 km above ground level. At ground level the average specific energy loss of the particles is about

2 Mev per 1 g/cm$^2$, i.e. approaches the minimum magnitude of any ionizing particles.

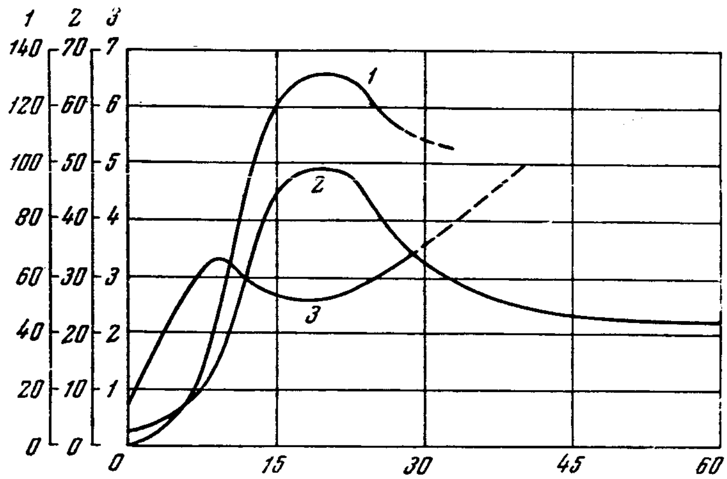


Figure 13. Total Ionization, Particle Count and Specific Loss of Energy of Particles as a Function of Altitude. Along the abscissa - altitude, km; along the ordinate: total ionization (curve 1), numbers of particles recorded by the counter (curve 2), average energy loss, in relative units (curve 3) (Tobias, 1956)

The distribution of ionization in the tissues of the body exposed to the effect of cosmic radiation (primary cosmic-ray particles) or to the radiations of the radiation belts surrounding the Earth is particularly complex. The pattern of the ionization inside the human body or the so-called "standard sphere" (a sphere with a substance of a specific weight 1, having a radius of 26 cm and weighing 75 kg) depends on the composition of the radiation, its energy spectrum, as well as on the objects and protective layers surrounding the "standard sphere". Thus, isodoselines characterizing the distribution of ionization inside the sphere will change all the time in accordance with the fluctuations of the radiation intensity, the movement of the "sphere" in space etc. For instance: re-entry of the "sphere" from the upper boundary of the atmosphere to the Earth will bring about a redistribution of the ionization

since the cosmic-ray particles will lose a part of the energy to overcome the resistance of the air layer above it (Schaefer, 1954, 1960, and others).

Freden and White (1959, 1960) placed blocks of emulsion plates into the nose part of a rocket which performed a ballistic flight, reaching an altitude of 1230 km (between 3 and 20° northern latitude), i.e., it penetrated into the lower layers of the inner radiation belt. Figures 14 and 15 show the energy spectrum measured by Freden and White as well as the spectrum of the path lengths. In this experiment the photo-emulsions were protected by material with a density of 6 g/cm$^2$. All protons with energies below 80 Mev were retained by this protection (the extrapolated curve is in dotted lines). A protection of 2 g/cm$^2$, which was proposed for large American spaceships, will pass all protons with energies exceeding 45 Mev (Schaefer, 1960).

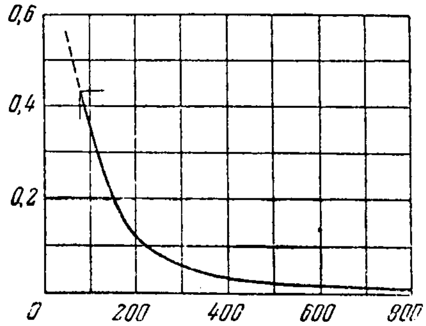


Figure 14. Energy Spectrum of Protons in the Inner Radiation Belt (According to Freden and White).

Along the abscissa - kinetic energy, $10^6$ ev; along the ordinate - protons/cm$^2$·sec·sterad·Mev (Schaefer, 1960)

By means of the curves reproduced in Figures 14 and 15 it is possible to calculate the ionization in each point of the "standard sphere" and to express the dose of radiation in terms of roentgens/hour. The highest dose is received by the surface layer of the "sphere" while fewer particles penetrate into the depth and, consequently, there the irradiation dose will be lower. In the surface layer the energy spectrum will change more drastically, while with increasing depth the changes will become more gradual (Figure 16).

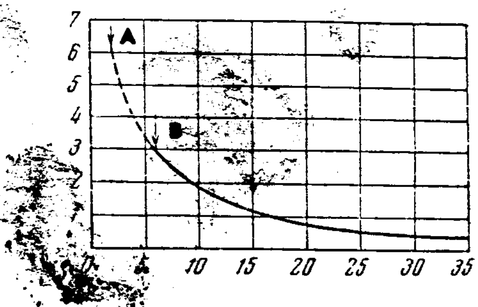


Figure 15. Spectra of the Path Lengths of Protons of Inner Radiation Belt. Along the ordinate - protons/$cm^2$·sec·sterad·g/$cm^2$; along the abscissa - path length of the protons in the tissue, cm; A - 2 g/$cm^2$ - minimum protection in a spaceship; B - 6 g/$cm^2$ - protection used in the experiments of Freden and White (Schaefer, 1960)

The hardness, i.e. the penetration power, of the proton beam as it passes through a certain layer of the medium, will become greater. This can be judged from the magnitude of the layer of 50% absorption, i.e. the shielding that can absorb half the power at this point. For X-rays the thickness of the layer for 50% absorption during passage through the shielding will at first increase sharply and then slowly, while a continuous increase and a total high value is characteristic for protons (Figure 17). The mean free path of the protons of the inner belt in air or in tissue equals about 120 g/$cm^2$. This characteristic of protons differs above different geomagnetic latitudes and also changes considerably during periods of solar flares. What has been said proves the extremely important role of the shape and size of the irradiated object, the protective shield surrounding it, as well as the composition of the spectrum of radiation which determine the total dose and the ionization pattern.

The maximum irradiation dose in Figure 16 equals 0.23 rep/hour. The dose is relatively small. However, the experiment of Freden and White was made in the lowest layers of the inner belt. If this dose is extrapolated to an altitude of 2200 km (assuming that the radiation intensity doubles every 100 km), then this will amount to an enormous dose - 120 rep/hour. However, it is necessary to bear in mind that such an extrapolation is not substantiated, since we are not justified in assuming that the energy spectrum of the particles remains the same at various altitudes (Schaefer, 1960a, 1960b).

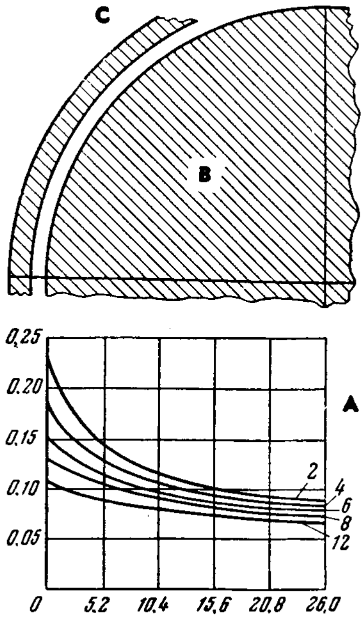


Figure 16. Ionization Dose Inside the Standard Sphere (Section of the Sphere). A - along the ordinate - rep/hour; along the abscissa - depth of the inner sphere $g/cm^2$; numbers at the right - shielding $g/cm^2$; B - object subjected to irradiation ("standard sphere"); C - shell of the spaceship (Schaefer, 1960).

## Principles of Physical Protection

The problems of protection from the effect of cosmic radiation are very difficult. This can be explained particularly by the inadequate study of the radiation fields beyond the atmosphere and inadequate information on its biological effects. The composition of cosmic radiation is complex and the principles of protection against various components of it differ and, therefore, the development of methods of protection must satisfy particular requirements.

For protection from charged nuclear particles, and particularly neutrons, it is advisable to use shields made of light substances, for instance, those which contain much hydrogen (water, paraffin etc.). As was mentioned above, in these the mean free path is lower. In addition, if shields of light substances are used, there are fewer possibilities of strongly ionized nuclear fragments being formed during collisions under the effect of cosmic rays.

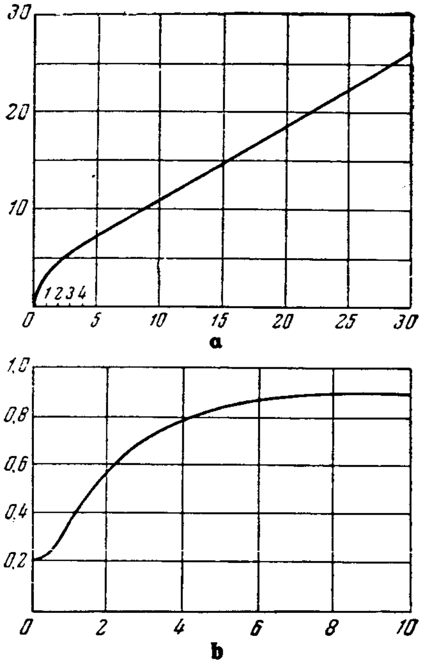


Figure 17. Depth of the Layer for 50% Absorption as a Function of the Depth of Penetration into the Medium of Protons and X-rays. Along the ordinate - layer of 50% absorption; along the abscissa - depth of penetration of radiation, g/cm$^2$; a - for protons of the inner belt (in tissue); b - for X-rays - 250 kev (in lead) (Schaefer, 1960)

As a protection from electromagnetic radiation (X-rays and $\gamma$-rays and for the absorption of electrons, heavy substances (lead etc.) are more suitable, since the absorption ability for these types of radiation depends on the atomic number and the weight of the substance.

In heavy substances, due to the effect of electrons, intensive bremsstrahlung is generated which possesses a high penetrating power and, under the bombardment of nuclei and high-energy protons, a certain number of penetrating $\pi$-mesons may form. In light substances (for instance of the beryllium type) these radiations form to a lesser extent.

For this reason it is proposed to make the protective shields of several layers, for instance, of a light material outside and a heavy material inside. The outer material will absorb charged nuclear particles and neutrons but will not influence the formation of bremsstrahlung. The inner material is intended primarily for absorbing electrons and electromagnetic radiations including the bremsstrahlung.

For absorbing characteristic radiations which form in the material, a multi-layer shield can be produced in such a way that each successive layer (in the direction from the outside to the inside of the spaceship) would be made of a heavier material than the one preceding it.

Protection against primary cosmic rays is extremely difficult due to their very high penetration power. Assumptions have been made (Tobias, 1956) that a water layer of a thickness over 1 m would be required. Inadequate protection may even intensify the effect of cosmic radiation due to the transition effect. The fuel on board the spaceship could be used as a protective shield. From the design point of view this problem is likely to prove extremely difficult. More recently, polyethylene has been suggested for use as a protective layer (Dye and Noyes, 1960). The idea of creating around the spaceship a powerful electromagnetic field that protects the capsule from particles appears almost fantastic at our present state of knowledge (Singer, 1958; Slater, 1960, and others).

However, the flux density of the primary particles is known to be low. Therefore, statistical and experimental biological estimation of the degree of danger of this radiation is necessary for determining the required and justified extent of extremely difficult protective measures against the effect of primary cosmic radiation.

Radiation from the periterrestrial belts has a much lower penetration ability than the primary cosmic radiation. Consequently, development of means of protection from this should in principle be a simpler problem. The high intensity (flux density) of these radiations, the high penetrating ability of protons of the inner zone and of the bremsstrahlung, the inadequate physical data on the periterrestrial belts, as well as the continuous fluctuations in intensity and composition which are difficult to predict, do not make this an easy problem to solve. For instance, it is still not known whether these radiation belts contain particles heavier than protons or what laws govern the fluctuations and the variations of the radiation field and the numerous other problems (Tobias, 1959 and others). Proposals have been made to launch special heavy satellites fitted with protection against radiation of the inner belt, which will move in this zone and, due to their mass, will absorb almost all the protons and will open up a way for later space flights (Singer, 1958; Slater, 1960 and others).

An effective means of protecting the crew of the spacecraft will obviously be the selection of a rational flight trajectory which would by-pass zones with high levels of radiation (Van Allen, 1960; Slater, 1960; Tobias, 1959, and others). It is essential to study carefully all changes in the radiation field in time and space.

The approximate radiation intensities in the periterrestrial belt, the velocity of movement of the spaceship during launching and the

assumed radiation dose received by the spaceship during a period of travel along two somewhat different trajectories are shown in Figure 18 (for a shield of 1 g/cm$^2$). The flight durations can be equal, for instance six hours, of which two hours will be spent in a zone of high intensity radiation. The cumulative radiation dose of the crew, protected by the light shield formed by the capsule walls (1 g/cm$^2$), will reach 10 rad (Van Allen, 1960).

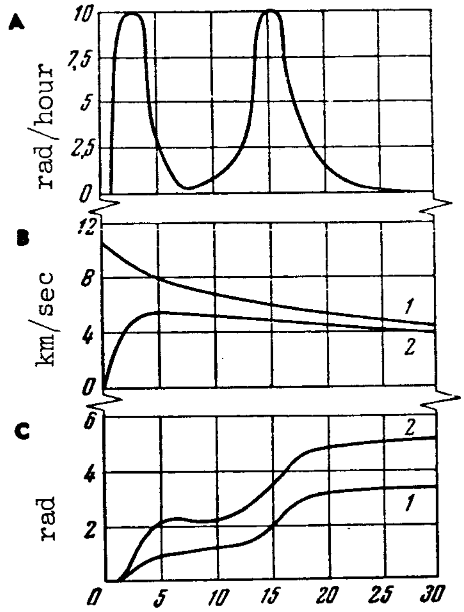


Figure 18. Radiation Dose of the Crew of the Spaceship in the Periterrestrial Radiation Belts. Plot A - curve of the radiation intensity as a function of the distance from the Earth; Plot B - curves of the velocities for two different launching trajectories; Plot C - radiation dose of the crew during the period of launching. Curves 1 - vertical trajectory; Curves 2 - elliptical trajectory (Schaefer, 1960)

During solar flares, the crew of a spacecraft protected by a shield of 1 g/cm$^2$ could receive a radiation dose 10,000 times the normal: during 15-20 min the cosmonauts might be subjected to the effect of 25-250 rad (Tobias, 1959).

The following preliminary conclusions can be made on the radiation danger during space flights.

1. It can be assumed that it is relatively safe to fly in satellites and spacecraft at altitudes between 250 and 600 km if the orbit has an inclination not exceeding $40^{\circ}$ relative to the equator (Van Allen, 1960).

2. Space rockets could leave [relatively safely] the Earth in polar regions.

3. Basically, 10 $g/cm^2$ will protect against the radiation danger in the outer belt; for protection against the aurorae a 1 mm thick layer of lead is sufficient, while a layer of lead a few millimeters thick is required for protection against bremsstrahlung.

A few millimeters of lead give full protection against electrons with energies of tens of kev and of bremsstrahlung caused by these radiations (0.7 $g/cm^2$ lead shield reduces the bremsstrahlung by a factor of two, while 4 $g/cm^2$ weakens it to 1% of the initial intensity). A 1 cm lead shield will reduce the $\gamma$-bremsstrahlung with energies of $10^6$ ev, produced by electrons with energies in excess of 1 Mev, by a factor of two only. Fortunately, this hard radiation is not of great importance.

4. Protection from protons in the inner belt has so far not been satisfactorily resolved.

5. At distances exceeding 10-15 Earth radii, the ionization effect of the cosmic radiation can be disregarded but its biological effect still requires study.

6. A rocket can cross the periterrestrial belt at a high velocity, during which the crew will be subjected to a radiation dose of several rem (Vernov, Chudakov, 1960; Van Allen, 1960; Tobias, 1959).

It may be considered advisable to provide in the spacecraft a special shielded box which the cosmonaut could enter just while passing through radiation belts or during sudden radiation danger. This would permit a great saving in weight, since the shielded box could have a considerably thicker protective layer while having a very much smaller volume. Furthermore, it is necessary to consider the possibility of partial shielding of individual parts of the body of the cosmonaut, which could increase the resistance of the body to radiation.

# Methods of Investigation of the Biological Effect of Cosmic Radiation[1]

Study of the biological effect of cosmic radiation is a difficult problem. For solving it various methods have to be used. It is possible to distinguish purely arbitrarily two main trends in the study of this problem, each of which has its advantages and disadvantages.

The first trend is the collection of information on the physics of cosmic space (in the first instance, on ionizing radiation) and subsequent synthesis of the results of study of the biological effects of the individual cosmic-ray components reproduced under laboratory conditions.

Information on cosmic-radiation physics should include the following basic characteristics: 1) what are the component (radiation) elements of the radiation field; 2) what is the intensity of the individual component elements (number of particles); 3) what is the energy spectrum of these radiations. This information is necessary for various regions of cosmic space. Furthermore, it is necessary to know how these characteristics vary with time. Unfortunately it is difficult to express the sum total of physical information on the radiation field (at a given instant for any zone of space) by a single quantity, for instance the rep, since high-energy dosimetry has so far not been adequately developed.

The difficulties are as follows. Firstly, the complex composition of cosmic radiation. Secondly, for high energies characteristic of cosmic rays, it is difficult to estimate the energy absorption in tissue, i.e. to calculate the absorbed radiation dose. Thirdly, it is not known how the energy which is absorbed by the tissue will become transformed, since for various types of radiations and various energies the absorbed energy will be transformed in different ways, and the quantitative ratio of the effects will differ (ionization, excitation of electrons, nuclear interactions, generation of bremsstralhung, X-ray and $\gamma$-radiation, etc.).

The main difficulty of estimating approximately the biological effect of cosmic radiation (when adequate physical information is available) is that its relative biological effectiveness is not known.

So far, the relative biological effectiveness of the heavy components of cosmic radiation cannot be determined under laboratory conditions, since no generators of very high-energy heavy particles are available. However, even if data on the relative biological effectiveness of

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[1]For convenience, by cosmic radiation in this communication we assume arbitrarily all types of ionizing radiations in space: primary cosmic radiation, radiation of solar origin, Van Allen radiation belts.

all components of cosmic radiation were available, this would hardly permit evaluating the biological effect of the total radiation by simply summing the assumed effect of the individual components. The position here is obviously far more complex (Birge, 1954; Evans, 1955, and others).

To collect adequate physical data, a relatively large number of experiments and much time are required. However, even with the most optimistic conclusions obtained by this method, preliminary biological tests, biological experiments with various living organisms, will have to be carried out if the safety of cosmonauts is to be guaranteed.

The second trend is the carrying out of extensive biological investigations on satellites and spacecraft.

By such methods data on the biological effects of cosmic radiation can be directly obtained. However, to obtain statistically reliable conclusions (particularly on the genetic effects), a great quantity of material is required, obtained in numerous experiments at various times for various zones of cosmic space and on a variety of biological specimens. This is particularly the case since we are dealing with discrete particles, the density of which is relatively low.

At present it is not possible to carry out a large number of biological flight experiments. Therefore, it is obvious that the two trends must be used to complement each other.

## The Relative Biological Effectiveness

The relative biological effectiveness of various types of cosmic radiations (particular corpuscular) depends on a number of factors. The density of ionization caused by radiations or the rate of energy loss of the particles plays a major part (Figure 19). An increase of the ionization density from two (minimum ionization) to 100 ion pairs per 1 $\mu$ of travel of the particle has relatively little influence on the relative biological effectiveness, which remains almost equal to unity. An increase of the specific ionization from 100 to 1000 pairs of ions per 1 $\mu$ increases appreciably the relative biological effectiveness (to 10-20). For instance, according to Shepherd (1953), the increase of the relative biological effectiveness is proportional to the logarithm of the ionization density. A further increase of the ionization density above 1000 ion pairs will lead to little change in the relative biological effectiveness and according to some data it may even decrease (Zirkle, 1954; Storer et al., 1957; Tobias, 1955, 1959; Coliez et al., 1959; Langham, 1959 and others).

Various authors estimate differently the relative biological effectiveness at ionization densities in excess of 1000 pairs of ions per

micron: from 2 to 30 (Shepherd, 1953; Storer et al., 1954, 1957; Krebs, 1954; Schaefer, 1954; Coliez et al., 1959; Tobias and Brustad, 1960). The International Commission of Protection against Radiation (1958) recommends assuming a relative biological effectiveness of 10-20 for a radiation producing an ionization density of 1500-5000 ion pairs/micron. A sharp increase of the relative biological effectiveness in the ionization density range from 100 to 1000 ion pairs per micron of the travel of the particle is in good agreement with both basic theories of the effect of ionizing radiation on living cells. According to the "target theory", at such an ionization density a critical concentration of ions inside the chromosome fiber is reached (1,000,000 ion pairs per 1 cm or 100 pairs per 1 $\mu$) and, according to the "indirect action theory", the concentration of hydrogen peroxide increases from zero to saturation (Tobias, 1955; Shepherd, 1957, and others).

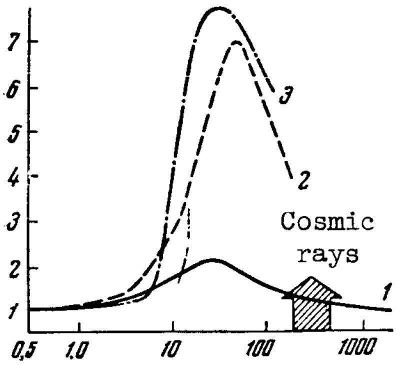


Figure 19. Dependence of the Relative Biological Effectiveness of Radiation on the Speed of Energy Loss of Particles (on the Ionization Density). Along the abscissa - rate of energy loss, kev/$\mu$; along the ordinate - relative biological effectiveness; curve 1 - mice; curve 2 - chromosomes AB; curve 3 - leguminous plants (Langham, 1959)

It is important to bear in mind that the relative biological effectiveness of any type of radiation depends to a large extent on the index being used, the function of the particular organ, and the period of time that has elapsed after radiation until the investigations are carried out (Table 5 and Figure 19). The relative biological effectiveness of a single type of radiation may be different for different biological specimens (Arsen'yeva et al., 1957; Dubinin, 1958, 1960; Glembotskiy et al., 1960; Khvostova and Nevzgodina, 1960; Krebs, 1954; Zirkle, 1954; Tobias, 1955; Storer et al., 1954, 1957; Langham, 1959, and others). Such factors as the strength of the dose of the radiation investigated as well as the features of the time distribution of the radiation dose are important (Schaefer, 1961; Deering et al., 1961).

Of great interest are the investigations carried out by Professor E.B. Kurlyanskaya et al. in 1962 when studying the biological effects of protons with energies of 660 Mev, produced on a 6 m synchrocyclotron of the Ob"yedinennyy institut yadernykh issledovaniy (United Institute for Nuclear Investigations), Dubna. These experiments, carried out on mice and rats, have shown that at densities of $10^8$ - $10^9$ particles per $cm^2$/sec, protons of such energies have a relative biological effectiveness equaling 0.5-0.6 if the evaluation is made on the basis of the lifetime and the changes in the peripheral blood. The relative biological effectiveness of these protons will equal 1 or more if the evaluation is based on the influence of the sexual glands, and the frequency of occurrence of malignant growths, a long time after irradiation. In this respect particular attention should be paid to work carried out recently in Soviet and foreign laboratories (Avrunina et al., 1961; Bonet-Maury et al., 1960; Deering et al., 1961; Zelmer and Allen, 1961).

Table 5. (Storer et al., 1954)

| Nature of the reaction caused by irradiation with thermal neutrons | Animal | Relative Biological Effectiveness |
|---|---|---|
| Lethality for 30-days $LD_{50}$ . . . . | Mice | 2.2 |
| Atrophy of spleen . . . . . . . . . . | " | 2.2 |
| Atrophy of thyroid . . . . . . . . . | " | 2.5 |
| Lowering of mitotic activity of skin . . . . . . . . . . . . . . . . | " | 2.2 |
| Atrophy of testis . . . . . . . . . . | " | 1.7 - 3.2 |
| Clouding of crystalline lens . . . | " | 6-8 |
| Influence on duration of life . . . . | " | 2 |
| Appearance of tumors . . . . . . . . | " | 2 |
| Lethality for 30-days $LD_{50}$ . . . . | Rats | 1.5 |
| Atrophy of intestine . . . . . . . | " | 1.7 |
| Uptake of $Fe^{59}$ by erythrocytes . . | " | 1.3 |

Some Features of the Biological Effects of
Ionizing Particles of Cosmic Radiation

The problem of the biological effects of heavy particles is a very complex one. The injury caused by neutrons and protons is more irreversible than that caused by X-rays. According to certain indices (clouding of the crystalline lens, genetic effects), neutrons and other particles appear to have a pronounced cumulative effect. In the case of repeated irradiations as well as in the case of increasing the duration of the period of observation after irradiation with particles, the relative biological effectiveness was particularly high, for instance it equalled eight for the crystalline lens (Evans, 1952, 1955 and others). According to other indices (lifetime and appearance of growths), this was not detected under similar conditions, and the relative biological effectiveness remained unchanged. From the greying of hair on mice, the effect of deuterons proved less pronounced, i.e. the relative biological effectiveness was lower than the effect of X-ray radiation (Chase, 1954).

The relative biological effectiveness of the genetic effect of the particles is particularly high. The ratio of the lethal and sterilization or genetic effects differ for different types of radiation. Although having relatively little influence on the ability to survive, particles may have a pronounced genetic effect (this has been studied to a greater extent for neutrons and $\alpha$-particles). As regards the effect of electromagnetic types of radiation, the opposite relation is characteristic (Arsen'yev et al., 1957; Dubinin et al., 1960; Breslavets, 1956). Neutrons cause greater changes in the chromosomes than in cell elements outside the chromosomes, whilst X-rays have the opposite effect. In the case of neutron action, diploid and polyploid type mutations may occur which do not occur during X-ray irradiation.

On determining the relative biological effectiveness of various types of radiation the doses used are of great importance. For instance, on changing over from small doses to large ones, the relative biological effectiveness of neutrons may change appreciably. The relative biological effectiveness and the qualitative features of the action of neutrons may change greatly depending on their energies (fast and slow or thermal neutrons). Thus, thermal neutrons, in contrast to fast neutrons, will produce more chromosome changes than gene changes. Even as regards the genetic effects, the relative biological effectiveness is estimated differently by different authors: from 2.5 to 13 and even up to 150.

Under the action of neutrons and protons, chromosome changes in cells will set in regardless of the state of development of the cell, in the same way as in response to X-rays these changes manifest themselves more easily during the phase of cell division (Evans, 1952).

The influence of oxygen on the mutagenic action differs in the case of different types of ionizing radiations. Under the action of particles, the oxygen effect (particularly the protective action of a reduced oxygen content) is not very pronounced, although the decomposition of water and the formation of hydrogen peroxide proceeds in the same way as during X-ray irradiation (Dubinin, 1958, 1960; Sidorov and Khvostova, 1960; Shchepot'yeva et al., 1961). During X-ray irradiation of tissue, a larger number of individual fluctuations can be observed than in the case of corpuscular radiation (protons, $\alpha$-particles, neutrons).

It is particularly important to note that fractionation of the irradiation dose of corpuscular radiation (prolonging and increasing the time intervals between irradiations), will reduce only very little their effectiveness (particularly as regards their effect on the gonads). Fractionation and prolongation of X-ray and $\gamma$-radiation are known to considerably weaken their effect (an effect which is widely used in radiation therapy). This indicates that corpuscular irradiation has a cumulative effect and that its action is relatively irreversible. It is very important to bear in mind the irreversible changes caused by corpuscular types of radiation when studying the biological effects of cosmic radiation, which has a very complex composition.

Many chemical and medical preparations, which effectively protect against the somatic effects of X-ray and $\gamma$-radiation will probably have little effect as regards corpuscular radiation, particularly against the genetic effects.

The interactions of various types of radiation energy are of definite importance. For instance, infrared rays may bring about potential changes (chromosome aberrations) caused by X-rays, and ultraviolet rays may aggravate the injury brought about by X-ray irradiation (Hollender, 1952).

It can be assumed that X-rays injure a larger number of cells in the body, but relatively slightly, while corpuscular radiation hits individual cells or groups of cells, but this is more pronounced and irreversible (Arsen'yeva et al., 1957; Breslavets, 1956; Dubinin, 1958, 1960, and others).

## Some Features of Ionization of the Body Tissue Caused By Cosmic Radiation Particles

The considerable difficulty in estimating the relative biological effectiveness of ionizing particles of cosmic radiation (protons, $\alpha$-particles and particularly of heavy multi-charged nuclei) consists in the peculiarities of ionization of body tissue.

X-rays and $\gamma$-rays have a diffuse action and cause ionization throughout the entire mass of the tissue. When irradiated with particles, energy is released discretely and for short periods at the place and time of passage of the particle, whereby the maximum energy per unit of travel of the particle is generated at the very end of the path, forming an ionization peak. For instance, an iron nucleus with an energy of 47000 Mev penetrates through 22 cm a thickness of body tissue, but the maximum ionization will occur only at the last 1000 $\mu$ of the path (Yagoda, 1957). The biological importance of the extremal, short duration release of a large quantity of energy has not been adequately studied (Campbell, 1954). The recommendations of the International Commission on Protection Against Radiation (1958-1959) and the U.S. Bureau of Standards (1959) do not give any bases for evaluating the dependence of the relative biological effectiveness on the pattern of ionization inside the body.

The problem of interrelation of the local and total effects of ionizing radiations has not been studied so far. Clinical experience of local X-rays or $\gamma$-radiation for therapeutic purposes has shown that to achieve local injury to tissue a high radiation dose is required compared to general (whole body) irradiation where the dose may be relatively small.

At an altitude of 21,000 m the tissue of living organisms is almost 55,000 times as dense as the surrounding air and, therefore, a relatively large number of particles will terminate their trajectory in it. It is important to know the distribution of the energy release by heavy particles in the human body as well as the changes in the biological effects of cosmic radiation when penetrating into the depth of the body and the probability of occurrence of ionization peaks inside the body.

The complicated pattern of ionization produced by cosmic rays inside the body or the "standard sphere" simulating it depends on the great variety of the penetrating particles, the spectra of their energies and the protective layer (the shell of the spacecraft, the air layer, the surrounding objects), etc. The composition of the cosmic radiation and its energy spectrum change continuously and this causes changes in the pattern of the ionization isodoselines inside the irradiated body (Schaefer, 1960).

In this way all the enumerated factors affect the distribution of the irradiation dose inside the body as well as the ratio of the doses at the surface and in the depths. In some cases the internal organs of a living organism are subjected to the greatest danger, while in others it is those close to the surface. Since in cosmic radiation the relatively low-energy component is predominant, the surface dose of an unprotected space pilot (inside the spacecraft) may reach very high magnitudes - tens of thousands of roentgens per hour. The high surface irradiation dose, as well as the low degree of protection of the tissues and the high

radiation sensitivity of the crystalline lens (particularly with respect to corpuscular types of radiation) present a real danger that it will become affected and a radiation cataract may form (Schaefer, 1960; Tobias, 1956).

Of great importance is the work of Yagoda (1956, 1957), who utilized a mockup of the human body and particularly of the head. Inside he simulated (in shape and specific gravity) the main anatomic formations. In the various sections of the "phantom" photoemulsion blocks were arranged and this enabled the study of the distribution of ionization doses inside the body (particularly the head) during experiments (flight as well as laboratory experiments).

Some authors consider that the danger of biological effects from cosmic radiation is not very real. It is true that at present the biological effects of the particles in the section of the trajectory where the specific ionization (ionization density) is very high are not known. However, in this section the particles expend only a relatively small part of their energy, while 83 to 96 percent of their energy is released at ionization densities, the biological effect of which has been studied.

The enormous energy (from $10^{18}$ - $10^{19}$ev) and penetrating ability of the primary cosmic-ray particles has been pointed out. However, in the energy spectrum of the particles the low-energy component is very predominant. Therefore, the average energy and penetrating ability of the particles will be very much lower than the above given maximum values. In addition, the heavy high-energy particles will in most cases terminate their path by collision with nuclei. After this, small fragments form (protons, $\alpha$-particles), the biological effects of which have been studied in great detail (Tobias, 1956).

So far, the effects of the primary cosmic radiation have been discussed. In the region of the periterrestrial radiation belts, the radiation dose will be much higher. However, the biological effect of these radiations has been investigated in greater detail. The relative biological effectiveness of the protons of the periterrestrial radiation belts will hardly exceed unity (Schaefer, 1960).

## On the Biological Effects of Heavy Cosmic-Ray Particles

The biological effects of heavy nuclei have been little studied, particularly in that section of their trajectory where they lose their energy rapidly and produce a very high ionization density, the so-called ionization peak. As a rough estimate, a rate of energy loss corresponding to 10,000 pairs of ions/$\mu$ can be considered as the threshold of formation of the ionization peak. Along the trajectory of the heavy particle a high ionization density is created. For instance, for a Fe nucleus in

a cylinder of a cell, 15 mm long and 50 $\mu$ in diameter, several thousand roentgens (about 15,000) are released in less than 0.000001 sec. Compared with the volume of the entire human body (thousand milliard cells), this is a relatively small value. According to the data of Chase (1954) and Simons (1958), at a distance of 100-120 $\mu$ around the axis of the track of the particle, the radiation dose reaches 100 rep. Considerably smaller values are shown in Figure 20 for the calcium nucleus (Schaefer, H. J., Golden, A., 1960). At a distance of 1 $\mu$ from the channel axis, this dose reaches 1000 rad; at 2 $\mu$ it is 100 rad, at 4 $\mu$ it is 10 rad.

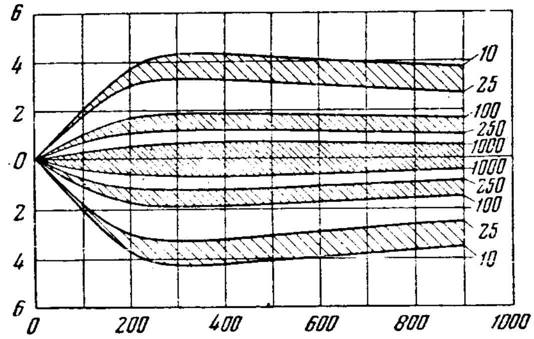


Figure 20. Isodoses of ionization of the tissue surrounding the final section of the trajectory of a heavy particle (calcium nucleus, Z = 20). Along the ordinate at the left - distance from the axis of the particle trajectory in $\mu$; along the ordinate on the right - dose of ionizing radiation (rad); along the abscissa - distance from the end of the trajectory of the particle, $\mu$. (Schaefer and Golden, 1960).

Attention is drawn to the fact that the radiation dose of cells decreases sharply in the direction from the axis to the periphery of the track formed by the particle. Therefore, cells situated at some distance from the track axis will receive a dose differing little from that caused by the natural background radiation of the Earth. In Figure 21 the magnitude of the radial distribution of ionization around the traces of a heavy nucleus (calcium) and $\alpha$-particles are compared with the dimensions of the cell of the human body and its chromosomes (Schaefer, 1958).

It can be assumed that after a stay of 24 hours in outer space (outside the periterrestrial radiation belts), 200-300 cells of the testicle will be affected by heavy nuclei ($Z > 2$). This represents only one millionth of all the embryonic cells of the body (Schaefer and Golden, 1960). Tobias calculated that in the case of a cosmic radiation dose equalling 0.07 rem in 24 hours, 0.0035 percent of all the cells of the body would be destroyed. This is considerably less than the number of cells which die daily in a body. The nerve cells are an exception and Tobias considers that the danger of affecting these is very real. For high-altitude

pilots the danger of biological damage due to cosmic radiation is extremely low. For a flight duration of 1000 hours per annum, the pilot will receive a dose below the maximum permissible dose for cases of chronic exposure to radiation (0.1 rem per week) (Tobias, 1956).

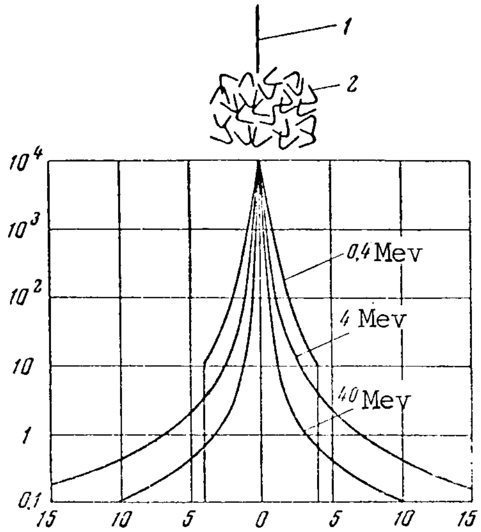


Figure 21. Radial distribution of the ionization of the tissue surrounding the track of a heavy particle with an atomic number Z = 20. Along the abscissa - $\mu$; along the ordinate - rem; 1 - track of an $\alpha$-particle, 2 - 48 chromosomes of the cell of the human body (Schaefer, 1958)

According to the calculations of Langham (1959), 50-60 heavy particles per hour will penetrate into the human brain in cosmic space. Schaefer (1954) assumes that about 100 heavy nuclei per hour terminate their trajectory with ionization peaks in the human body. On the basis of experiments with photoemulsions, Yagoda (1957) arrived at the conclusion that 28 heavy particles ($Z \geq 6$) produce ionization peaks in each 1 $cm^3$ of tissue per 24 hours at the upper boundary of the atmosphere, 11 at an altitude of 36 km and only 1 at an altitude of 24 km. Thus, the number of ionization peaks received by the tissue of the face and eyes per 24 hours during cosmic flight is about 20.4. The ratio of lighter nuclei (C-N-O-F-Ne) to heavier nuclei (from Na to Fe) at an altitude of 36 km is approximately 3:1.

Much statistical work is required to evaluate the true danger from individual cosmic-ray particles to various organs and tissues. As is well known, the flux density of these particles is not high. Their

biological effect can vary greatly, depending on the affected group of cells. It is necessary to know how the failure of various groups of cells may affect the functioning of an organ, the tissues and of the entire body.

Connective tissue cells, although highly sensitive to radiation, will probably recuperate easily after being affected in view of their very fast regeneration. The situation is more complex as regards the highly differentiated nonregenerating cells (nerve cells, cells of the parenchymatous organs, etc). This is particularly the case if the cells are highly specialized and there is no possibility of compensating their functions by adjacent cells. For instance, if the receptors of the retina are affected, this may lead to microscotoma, if the cells of the audio receptor are affected this may lead to loss of sensitivity to a certain tone, if some centers in the brain are affected, (particularly at the floor of the fourth ventricle) - to disruption of functions which are vital for the process of living, if groups of pigment-forming cells of the follicles of the hair are attacked, it will lead to irreversible greying (Schaefer, 1958b, 1958B; Chase, 1954; Chase and Post, 1956 and others) etc.

Views have been expressed that if one or a group of embryonal cells are affected at an early stage of their development, their specialization may become degenerated and impede the development of the organism. These assumptions were based on experiments with mechanically damaged cells of embryos of lower animals. However, in embryos of highly organized animals the cells are considerably less differentiated and they have a higher ability of interchanging, compensating and regenerating. To influence the development of a human embryo it would be necessary to damage a large number of cells or the damage would have to occur at a very early stage of development. In this case, however, the most frequent occurrence will be that the embryo will die. Unfortunately, all the views expressed are tentative, and solution of the problem requires a large number of laboratory and flight experiments (Schaefer and Golden, 1960; Russel, 1954, Pobedinskiy, 1961).

## On the Genetic Effect

The problem of the possible genetic effect of cosmic radiation attracts particular attention. As is well known, considerably smaller radiation doses are required to produce a genetic than a somatic effect. The view is relatively widely accepted that there is no threshold from the point of view of the genetic effect, i.e. there is no ionization dose below which it would be impossible to detect a genetic effect on a sufficiently large population of organisms. If the doses are small, only the statistical probability of the occurrence of a genetic effect among people subjected to irradiation will be reduced. In individual cases a

single-energy quantum, a single particle (for instance, an electron) suffices for producing a genetic change (Pugsley et al., 1935; Newcombe, 1960, and others). Thus, determination of the threshold dose produced by the genetic effect is at present limited by the potentialities of the methods of detecting a statistically reliable genetic effect (Glembotskiy, Abeleva, Lapkin, 1960, and others).

The work of Soviet scientists has shown that there is a high genetic danger even in the case of small doses of ionizing radiations. The sensitivity of highly organized animals is higher than for animals at lower stages of evolution (for monkeys it is twice as high as it is for mice). Experiments carried out on human tissue cells showed that a dose of 5 to 10 r will bring about a statistically reliable doubling of the number of affected chromosomes.

The relative biological effectiveness of corpuscular types of radiation is particularly high for the genetic effect. Chemical preventive agents which protect against the somatic effects of the ionizing radiation are not always sufficiently effective to prevent genetic damage (Dubinin, 1957, 1958, 1960; Nuzhdin, Shapiro et al., 1955; Tinyakov, 1960; Dubinin, Arsen'yeva, Kerkis, 1960; Dubinin, Kerkis, Lebedeva, 1960; Sidorov and Khvostova, 1960; Evans, 1952; Muller, 1952, 1954; Russel, 1954).

The danger of the genetic effect of small doses of chronic irradiation with ionizing radiations and new data on radiation genetics have induced the International Commission on Protection Against Radiation to reduce the maximum permissible dose (Recommendations ... 1955-1959). It is necessary to underline again the undoubted genetic damage to people of even minimal radiation, since with rare exceptions the resulting mutations are unfavorable, and pathological.

In determining the genetic danger of space travel it is of great importance whether the flight is carried out by individuals (cosmonauts), or by a large number of people, which may be of genetic importance to society as a whole. It is important to know the ages of the cosmonauts and the prospect of their having offspring. It is possible that certain measures should be taken to prevent the birth of offspring within a certain period after the flight, governed by the stages of the spermatogenesis which may have suffered under the effects of radiation. In estimating the genetic effects of cosmic radiation, it is necessary to take into consideration the danger to future generations (drop in the birth rate, stillborn babies, pathological effects in the offspring, etc.) as well as possible cytogenetic changes in the irradiated organism leading to a shortening of life as a result of blood disease, malignant growths and other pathological phenomena (Krebs, 1950, 1952a, 1952b, 1954, and others).

In studying the genetic effects of cosmic radiation it is necessary to consider also the possible genetic changes in plants (for instance, the water plant chlorella) and other living organisms intended for ensuring the necessary living conditions for the cosmonaut in space. If mutations occur in these plants or animals during the flight, the properties of the organisms may change (growth, multiplication, etc.) which will disturb the balance of the closed ecological system of the spaceship.

In taking into consideration the genetic effects of space flight, it is necessary to bear in mind that future space flights may take a very long time (for instance, flight to other star systems may last for several generations of cosmonauts flying in the ship). It is known that the genetic effects caused by fractionated ionizing irradiation (as well as X-rays and $\gamma$-rays), linked with the irreversibility of changes in the gonads, are highly cumulative (Nuzhdin, Shapiro et al., 1959, and others). The cumulative properties of the biological effects are still more pronounced for corpuscular types of radiation. Therefore, one should anticipate the full cumulative effect of cosmic rays for the entire flight duration (Dubinin, 1957, 1958, 1960, and others).

## On the Combined Effects of Cosmic Radiation and Other Flight Factors

Any living being will be subjected during flight in space to an entire range of factors (ionizing radiation, accelerations, vibrations, weightlessness, changes in barometric pressure and gas composition, numerous emotional-psychic effects, etc.) which will act on him in a variety of combinations and sequences.

To estimate the biological effects which are to be anticipated, it is necessary to know the sequence and durations of the effects of the individual factors (including various types of ionizing radiations) during travel through various zones of cosmic space. Due to the radiological nonuniformity of cosmic space, irradiation of the space pilot will have a peculiar time distribution, which is of great importance.

The importance of the combination of the various factors can be judged from the following example. In the experiments with Layka on the second artificial earth satellite (Chernov and Yakovlev, 1958), as well as in some other work (Beckh, 1959, and others), interesting information was obtained on the peculiar physiological effect of accelerations which are followed by weightlessness and vice versa, accelerations that follow weightlessness.

Of great importance is the problem of the combined effect of ionizing radiation (particularly cosmic radiation) and of other flight factors. Here, changes in radiation sensitivity (particularly with respect to the

genetic effects) as well as changes in stability of response to other factors can be anticipated. Even the first investigations in this direction have confirmed previously expressed assumptions (cytogenetic effects of vibration and irradiation). Unfortunately, problems of the combined effect of radiation, accelerations, vibrations, barometric pressure and other factors have not been adequately studied (Sisakyan, 1960, 1961; Arsen'yeva et al., 1961; Glembotskiy et al., 1961; Parfenov, 1961; Ganshina, A. N., 1961; Kimelsdorf and Newsom, 1953; Newsom and Kimelsdorf, 1954; Taylor, 1960; Konecci et al., 1956, and others).

Comprehensive estimation of the numerous factors affecting space flight is required: living conditions in the capsule of the spaceship (feeding, water supply, air regeneration, work and rest, emotional and psychic, sanitary-hygienic conditions, etc.), and the numerous unusual external factors which will act on the cosmonaut as well as various individual features of the cosmonaut (sex, age, individual radiation sensitivity, etc.). The importance of the enumerated factors from the point of view of being affected by radiation is well known in modern medical radiology. For the purpose of selection of future astronauts it would be very useful to have a convenient, fast and safe method of determining the individual radiation sensitivity of subjects.

Soviet scientists have shown that the central nervous system is highly sensitive to the effect of ionizing radiation (Livshits, 1961; Lebedinskiy and Nakhil'nitskaya, 1960, and others). Under the conditions of high emotional and psychic stress pertaining during flight, the effect of cosmic radiation may manifest itself in the functioning of the central nervous system and in the working ability.

Biologists and biophysicists have not yet studied the possibility of formation of induced radioactivity in the body of the astronaut, the foodstuffs and the objects surrounding the astronaut etc. under the influence of high-energy particles (Campbell, 1954; Eugster, 1957, and others). The danger of induced activity of the tissues of the body lies in the fact that these may become sources of radiation which act continuously on the body for a long time after the flight (for durations depending on the half-life of the isotopes formed).

The problem of the biological effect of the magnetic field requires further study. Work has been carried out which indicates its influence on the development of plants (Krylov, 1961), its action upon condition reflexes and upon the dynamics of the fundamental nerve processes in the brain (Kholodov, 1961). However, a number of investigations of the biological effects of the magnetic field yielded negative results (Eiselein et al., 1961; Beischer, 1962, and others). The problem of the effect of microwaves on the body has also not been adequately studied (Presman et al., 1961). This problem is of great importance in view of the rapid progress, particularly in the field of astronautics.

It is also necessary to bear in mind the dangerous effects of cosmic rays on instruments and apparatus fitted to ensure conditions of living and flight safety. Views have been expressed in the literature on this subject, for instance, concerning possible effects on solar batteries (Newell, Naugle, 1960).

The prospect of designing engines based on atomic propulsion for future spacecraft will bring up new problems of protection of the crew from the radiations of the reactor. As regards spaceships, this is a difficult problem which has attracted the attention of designers and doctors in recent years (Barnes, 1960; Konecci and Trapp, 1959; Conner, 1960; Ebersole, 1959; Pickering, 1960; Reitz, 1960; Talbot, 1952; Air University Quarterly Review, 1959, Vol. II).

## Some General Biological Problems

Investigation of the biological effects of cosmic radiation involves a number of important methodological and general biological aspects.

Cosmic rays that reach the surface of the Earth act continuously on all living beings as part of the natural radiation background. The biological effect of the natural radiation background (Siventsov, 1960; Israel, 1957, and others) attracts the attention of biologists. The natural radiation background on the ground, its individual components and the irradiation of the gonads of man are listed in Table 6 (Dubinin, 1957).

Table 6

| Sources of Natural Radiation | Dose, acting on the gonads per annum, rads |
|---|---|
| External radiation background | |
| Cosmic rays (at sea level) . . . | 0.028 |
| $\gamma$-radiation of the Earth . . . . | 0.043 |
| Radon in the air . . . . . . . . | 0.001 |
| Internal radiation background | |
| Potassium-40 . . . . . . . . . . . | 0.020 |
| Carbon-14 . . . . . . . . . . . | 0.001 |
| Radon . . . . . . . . . . . . . . | 0.002 |
| Total per annum . . . . . . | 0.095 |
| Cumulative dose per generation . . | 2.85 |

In the 1920s and 1930s the problem was discussed in literature on whether cosmic rays which reach the surface of the Earth are one of the factors bringing about evolution. Numerous researchers considered and still consider that from the time of formation of life on Earth and the entire history, cosmic rays have affected living organisms and represent one of the most important (if not the main) factors in evolution, by bringing about the basic mass of natural "spontaneous" mutations (Kol'tsov, 1935; Hadson, 1935; Joly and Dixon, 1929; Babcock and Collins, 1929; Hurst, 1932; Thomas, 1936; Tobias, 1959; Wesley, 1960, and others). Others, after carrying out calculations of the assumed genetic effect of radiation, have shown that at ground level cosmic rays can cause only 1/1000 of the natural mutation (about 3 percent in high mountains) and consequently cosmic rays cannot be considered as an important factor in evolution. However, the very fact that cosmic rays can, in principle, cause mutations in beings on Earth has not been disproved by them (Meller, 1935; Muller and Mott-Smith, 1930; Delbruck, Timofeev-Ressovsky, 1936; Frizen, 1936; Engelstad, Moxness, 1934; Spiers et al., 1960; Brues, 1961).

From the results of experiments on biological objects placed deep in the ground (Babcock and Collins, 1929; Engelstad and Moxness, 1934), using various shields (Tzaschel et al., 1954), as well as in balloons (Frizen, 1936), the respective authors have been able to draw conclusions on this controversial topic. These experiments cannot be considered sufficiently convincing in view of difficulties in using the method, which were not resolved in the works referred to.

Taking into consideration the relatively low dose of the ionizing effect of cosmic radiation beyond the atmosphere (10-15 billion per 24 hours) H. Miller (G. Meller) (1935, 1952) considered that this can hardly represent a genetic danger in future flights. At that time the existence of the powerful radiation belts surrounding the Earth and the periodic increases in intensity of cosmic radiation associated with the activity of the Sun and other factors were not known. All this requires much caution in estimating the radiological, (genetic) danger attached to space flights.

As long ago as 1934 the basic problems on the possible biological (particularly genetic) effects of cosmic radiation were formulated and concrete spheres of investigation were outlined at the All Union Conference on Stratosphere Study in papers of the Soviet scientists L. A. Orbeli, N. N. Kol'tsova, G. A. Nadsona, B. L. Isachenko, L. P. Perets and others, as well as the American scientists H. Miller (G. Meller).

Space biology should solve two basic problems:

1) Study of conditions of living in space and of the genetic effects of space flights;

2) study of the existence of living matter and organic substances in space and on other celestial bodies.

Solution of these problems will provide scientific proof of the extended development and advanced philosophical concepts of dialectic materialism (Zhukov-Verezhnikov, Yakovlev and Mayskiy, 1959, 1960).

In studying the conditions of life in space, methods which permit detailed study of the biological characteristics and particularly of the genetic effects of space flights, primarily of cosmic radiation, are of great importance, in addition to the physiological methods of recording the living activity of complex organisms. For this purpose the use of single and multicell organisms and biological "substations", study of their metabolism, fertilization, multiplication, overall survival time and of other functions opens up great prospects. It is emphasized that for studying the biological effects of cosmic rays it is important to utilize various representatives of the animal world which are on different phylogenetic lines and levels of evolutionary development. Only an extensive collection of methods and objects of investigation will permit solving this extremely complex problem (Sisakyan, 1960, 1961).

The small dimensions of the microbiological and cytological specimens, the comparative ease of ensuring the necessary conditions of living and the short time between successive generations make them very convenient specimens for obtaining extensive statistical material, which is particularly important in genetic investigations. The relatively low radiation sensitivity of most microscopic specimens is more than compensated by the colossal number of these objects. The consequences of being hit by a single heavy cosmic-ray particle, which are extremely difficult to detect in a multicell organism, can be seen very clearly from the genetic changes in colonies and groups of cells of microbiological and cytological specimens (Sisakyan, 1960; Zhukov-Verezhnikov et al., 1959, 1960; Zhukov-Verezhnikov, 1961).

The prospect of utilization of microbiological and cytological specimens in space investigations, as well as the problem of finding various forms of microorganisms in space and on other celestial bodies has brought about the emergence of a new branch of science - cosmic microbiology (Zhukov-Verezhnikov et al., 1959, 1960; Lederberg and Cowie, 1958; Vishniac, 1960).

Of great theoretical interest is the problem of the existence of life beyond the Earth. Although this problem has occupied the minds of scientists for a long time, it is only now becoming possible to change over from speculative arguments to concrete investigations. Some investigators (Arrhenius, 1900, 1912; Lederberg and Cowie, 1958, and others) have assumed and still assume the possibility of existence in space of spore forms of microorganisms. Microorganism spores are known to be very

resistant to unfavorable external effects. It is possible that they are carried from one celestial body to another inside meteoric particles. It can be assumed that if the trajectories of movement of these particles are favorable (if they bypass zones of intensive radiation) some spores conserve their ability to live in spite of the long flight duration and the enormous velocity of fall onto the celestial body.

Celestial bodies may have the necessary conditions for the existence of microorganisms. For instance, Lederberg and Cowie (1958), Lederberg (1960), Sagan (1960a, 1960b) and others, consider that for such microorganisms the conditions of life on the Moon under the dust layer are favorable. The microorganisms from cosmic space can adapt themselves to unusual conditions due to the development of protective mechanisms, a sharp reduction or change in the form of interaction with the surrounding medium (Zhukov-Verezhnikov et al., 1960; Vishniac, 1960).

The work of the Soviet astrobotanist G. A. Tikhov (1959, 1960) conclusively shows the possibility of existence of life (plants) on planets nearest to the Earth. More recently, there has been a great intensification in the study of the possibility of existence of various forms of life on other celestial bodies, a search for methods of detecting signs of life (for instance by using spectrographic, polarization and other astrobiological methods of investigation), as well as an intensification of the study of substances which are enclosed inside meteorites (particularly in carbon meteorites). The concept of Academician Vernadskiy on the biosphere (1960) has been further developed in a new branch of knowledge referred to as exobiology (Sisakyan, Gazenko, Genin, 1961; Imshenetskiy, 1962; Lederberg, 1960; Sagan, 1960a, 1960b; Dollfus, 1960; Huang, 1960; Shapley, 1960; Young and Johnson, 1960; Keosian, 1960; Grossenbacher, 1960; Strughold, 1960; Calvin and Vaughn, 1960; Bracewell, 1960).

Investigation of the problem of the existence of living matter and organic substances in cosmic space as well as the possibility of such matter being carried from one celestial body to another will present an important contribution to the solution of biological and philosophical problems relating to the origin of life - it is sufficient to bear in mind the "panspermia" theory of Arrhenius (1900-1912). Comparison of forms of life found in cosmic space with forms of life found on Earth will permit the clarification of the nature of formation and development of life in the Universe (convergent or divergent evolution). There is reason to believe that if life is detected outside the Earth, then its forms will confirm the unity of the laws of development of living matter in the Universe, and the unity of the laws of the creation of the world (Zhukov-Verezhnikov, Yakovlev, Mayskiy, 1960).

It is necessary to bear in mind that in order to detect living matter in space, great difficulties have to be overcome as regards methods

of trapping meteoritic particles. The difficulty lies in the fact that the latter move with enormous velocities and that they explode and cease to exist as soon as they come in contact with any other object (Zhukov-Verezhnikov et al., 1959, 1960).

In conjunction with space microbiology there is also another practical task, namely, the prevention of bringing from other celestial bodies to the Earth unusual (possibly very harmful) microorganisms, as well as contaminating other celestial bodies with terrestrial forms of microbes. For this reason the Soviet rocket which reached the Moon was disinfected prior to being launched.

## Study of the Biological Effects of Cosmic Radiation in Ground Experiments

In addition to flight experiments, laboratory experiments play a certain role in studying the biological effects of cosmic radiation.

Already at the end of the 19th century the opinion was expressed that the biological processes on Earth depend on the activity of the Sun and other phenomena in the Universe (Arrhenius, 1900). From 1915 onwards the influence of the "explosions" and cyclic activity of the Sun on the functional state of the nervous system, the state of health and the mortality of man as well as the living activities of various organisms were studied by A. L. Chizhevskiy (1930) and other authors.

Over a long period, Brown, Webb, Bennett (1958) compared the fluctuations in the ionization intensity of the Earth brought about by cosmic radiation at the Earth's surface with the life activities of biological organisms. In some cases they noticed activation of life processes under the influence of increased radiations, in other cases they noticed a decline in these activities. However, there was always a clear cut synchronization and interaction between the biological activities and the intensity of cosmic radiation. Kaulbersz et al. (1958) detected shifts in the biochemical reactions and the number of leucocytes of the blood during the total eclipse of the Sun in 1954.

A number of authors reported that they found a relationship between biochemical and physicochemical serological reactions (particularly sedimentary) and cosmic factors, primarily the intensity of cosmic radiation (Shul'ts, 1961; and others).

Even earlier, some researchers, for instance Buettner (1952) cautioned against rash conclusions in this respect. He pointed out that the dependence of vegetative reactions (particularly the Takat reaction) on the activity of the Sun is difficult to explain by the effect of cosmic rays. This dependence may be due to other, hitherto unclarified, factors.

The material presented in the work of proponents of the dependence between the activity of the Sun and biological phenomena requires verification and further study.

It is necessary to compare the results of experiments carried out on biological specimens under various conditions: 1) at sea level; 2) with maximum protection from the effects of cosmic rays (deep under the surface of the ground or water). In this connection, it is necessary to bear in mind that at greater depths the natural radioactivity of rocks may sometimes be high although the penetrating ability of this radiation is small. It is necessary to select a location in which the surrounding rocks have a low radioactivity and to ensure the appropriate shielding of the biological specimens; 3) under conditions of intensified cosmic radiation: a) in high mountains, in air balloons, rockets, etc., b) under various shields (for instance, lead) which intensify the effect of cosmic radiation due to the transition effect.

Obtaining reliable data from ground experimental studies of the biological effects of cosmic radiation involves great difficulties. The biological specimens must be provided with the necessary conditions of life (temperature, humidity, etc.) which are equal in every respect except for the cosmic radiation. In view of the low total dose of ionizing radiation, a very large quantity of statistical material and very long experimental durations with subsequent evaluation of many successive generations will be required (Eugster, 1953, 1955).

Babcock and Collins (1929) placed Drosophila in a tunnel 140 m deep, but the natural radiation background there proved to be higher (due to the radioactivity of the rocks) than at the ground surface. As a result, more mutations were observed in the experimental Drosophilae than in the comparison standards. Engelstad and Moxness (1934) kept mice deep in a shaft for one year. They failed to find any difference between these mice and the control ones. In view of the enormous penetrating ability of the individual components of cosmic rays, Eugster (1957) placed experimental specimens into air balloons and the specimens serving as controls into very deep tunnels (under the surface of the Earth at a depth of 2300 m along the vertical and 10 km along the horizontal).

Tzaschel et al. (1954) placed bacteria (Bac. subtilis) under lead and timber shields. Due to the transition effect, lead shields intensified the action of cosmic rays. In bacteria under a lead shield the investigated effects of cosmic rays (the influence on the respiration of the bacteria) were most intensive. Rajewsky et al. (1936) also observed an increase in the frequency of mutations in fungi (Bombardia lunata Zickleri) which grew under a lead shield.

V. Sh. Kamalyan (1958) observed that in the families of the personnel of a high mountain laboratory, located at an altitude of 3200 m above

sea level, primarily girls were born during the first period of their work at high altitudes. In experiments with animals living in mountains it was also observed that preferentially females were born. V. Sh. Kamalyan considers that these data may be due to a number of factors, including intensified cosmic radiation at higher altitudes. It is interesting that out of six puppies of the dog "Strelka" born in the first litter after her flight, five turned out to be female and in the second litter of four puppies there were three females. Obviously, however, a great deal of statistical authenticity and caution needs to be observed when deriving conclusions.

V. I. Danileyko (1961) observed a temporary focal depigmentation of the hair in mice which had stayed over a long period in mountains at an altitude of 2200-3900 m above sea level (El'bruss Expedition). The number of erythrocytes proved higher in mice which were at this altitude in an open cage than in mice which were in lead-lined cages. V. I. Danileyko concluded that radiation under mountain conditions influences the hemopoietic function.

Eugster (1955) proposed a number of procedures for studying the biological effect of cosmic radiation and applied these in experiments in balloons, and rockets, as well as in laboratory experiments on the study of the effect of various types of ionizing radiations. The feature of these methods consists in the fact that two indices are studied simultaneously: the biological and the physical (photoemulsion). These experiments permit comparison of the track of the particle (type, energy and direction of travel of the particle) with the biological effect caused by it. The photoemulsion layer is placed on the biological specimen (seed, skin, etc.) either directly or by using a thin interlayer (cigarette paper). In other experiments biological specimens (for instance, the eggs of crustaceans - Artemia salina) are mixed with photoemulsion. In a third series of experiments a photoemulsion or a special dye, which changes its color under the effect of ionizing radiation, was introduced subcutaneously into human or animal skin flaps which were to be subjected to irradiation (intra-vitam stain method).

Use of special chemical substances (folin-phenol reagents, triphenyltetrazole chloride), which change color under the effect of high ion concentrations ($10^4$ ion pairs per $1\,\mu$) enabled Eugster (1952) to carry out special investigations on the chemical effects of cosmic radiation.

Yagoda (1957) developed a technique in which a photographic plate with two parallel layers of photoemulsions (layer thickness - $600\,\mu$) was immovably attached above the head of the animal to be subjected to irradiation under flight or laboratory conditions. The plate permits recording the location and the angle of entry of the particle into the skull, which during subsequent histological investigations considerably

facilitates locating the tracks of particles which have penetrated into the brain. According to the calculations of Yagoda, it is necessary to inspect on the average 500 $cm^2$ of histological preparations in order to detect the section through which even one particle passed over a period of several days.

Chase (1954) and Chase and Post (1956) proposed utilizing the greying of hair on the skin of the experimental animals (for instance, in black mice of the line $C_{57}$) as an index of the biological effect of cosmic-ray particles and corpuscular types of radiation generated under laboratory conditions. As is well known, a certain dose of X-rays and $\gamma$-rays brings about greying and loss of hair. This method has potentialities in studying the effect of corpuscular radiation. In this respect the skin with the hair plays the role of a "photoemulsion" which permits investigating the discrete effect of the particles. In the hair follicle only a small group of cells is associated with the pigmentation function. Acting on these cells, radiation causes a greying of the hair. The radiation sensitivity of the cells depends on the functional stage of the follicle (quiet or active follicle; maternal cells which are in a state of division). Knowing the dimensions of the follicle and of the group of its radiosensitive cells (30-40$\mu$), the depth of their location and the distance between the individual follicles (100-140$\mu$), as well as the radiation dose (in roentgens) which causes a greying or loss of hair in various stages of activity of the follicle, it is possible to judge the intensity of ionization and its redistribution along the track of the passage of the particle through the tissue. The given data on the distribution of the radiation dose in the tissue surrounding the track of the particles are based to a considerable extent on the results of the experiments of Chase. To simulate the effect of heavy cosmic-ray particles, particularly on the ionization peak section, Chase (1954) irradiated the skin of mice with X-rays and deuterons (dose 1000-35000 rep) through apertures of 1 mm, 100 and 50$\mu$ in diameter.

A number of authors have carried out a comparative study of the biological effects of X-rays and $\gamma$-rays, electrons, protons, deuterons and $\alpha$-particles of various energies accelerated by means of cyclotrons and linear accelerators (Fedorova, Avrunina, 1961; Yanovskaya, 1961; Kurlyandskaya et al. 1962; Tobias, 1952, 1959; Birge, 1954; Storer et al., 1954, 1957; Chase, 1954; Tobias and Brustad, 1960; Bonet-Maury et al., 1960; Chase et al., 1961; Deering et al., 1961; Zellmer and Allen, 1961).

The maximum energy of these particles produced by means of modern accelerators is at the lower boundary of the energy of the primary cosmic-ray particles. Particularly low is the energy of particles emitted by radioactive isotopes. Thus, in these biological investigations the effect of the particles was relatively a surface one. One must admit that

many contemporary views on the problem of the biological effect of the heavy components of cosmic radiation are only conjectural and are based on extrapolation of data obtained under laboratory conditions with radiations representing a very remote simulation of cosmic radiation.

Attempts have been made to investigate, by means of special accelerators, the effect of nuclei of carbon, oxygen, neon and argon with energies up to 10 Mev per nucleon. Particles with such an energy penetrate into the tissue to a very shallow depth only (for instance, carbon penetrates to 500 $\mu$), which permits studying the effect of nuclei only on unicellular organisms. Accelerators are required which would yield particles with energies of 1 Bev per nucleon (Tobias, 1959).

## Study of the Biological Effect of Cosmic Radiation in Flight Experiments

Flight experiments provide favorable possibilities for studying the biological effects of cosmic radiation. The intensity of cosmic radiation in mountains at an altitude of a few kilometers is only 2 to 3 times higher than at sea level (where the intensity is 0.1 mrad/24 hours); at an altitude of 20-30 km the intensity of cosmic radiation is 150-200 times (15 mrad/24 hours and more) higher than at sea level. However, there are very few primary cosmic-ray particles at this altitude.

At the upper boundary of the atmosphere the total intensity of cosmic radiation (the ionizing effect) is no higher than at an altitude of 20-30 km. However, at the upper boundary of the atmosphere it is possible that the biological effect of primary cosmic radiation may differ appreciably from the biological effect and the relative biological effectiveness of the secondary particles.

Air balloons permit exposure of biological specimens at altitudes of the order of 30 km for long periods (24 hours and more). High altitude and ballistic rockets can lift biological specimens to the boundary of the atmosphere and beyond it. However, the duration of exposure in this case is very short (15-20 min). Artificial earth satellites ensure a sufficiently high and long flight duration. The possibilities are particularly favorable for extensive and comprehensive study of the consequences of space travel if the living organisms are returned to Earth after the flight.

As long ago as 1934, biological experiments in air balloons were planned in the Soviet Union. In 1935 an experiment was carried out with Drosophilae which were placed in the stratosphere balloon USSR-1, this rose to an altitude of 15,900 m (Frizen, 1935). No increase was observed in the number of mutations of these flies after the flight. From these results the conclusion was reached that cosmic rays cannot be considered as being an important factor in the evolution of organisms on Earth.

In 1935 a successful biological experiment was carried out in the USA on the aerostat "Explorer II". In the gondola of this aerostat Drosophilae and spores of fungi were placed, which withstood satisfactorily the flight conditions. By means of special equipment air specimens were taken at the high altitude, and in these microflora were detected (Meier, 1936; Jollos, 1936; Rogers and Meier, 1936; Briggs, 1936).

In 1946-1947 in the USA, V-2 rockets with spore fungi and Drosophilae were launched and in 1950-1952 rockets with lower-order monkeys and mice. The flights were vertical and the altitude reached was 100 km. As a result, the biological specimens were exposed to conditions of cosmic space for a very short time. Most of the experiments (particularly up to 1951) were unsuccessful and the animals perished.

Launching of air balloons with biological specimens continued in 1951-1952 and 1953-1957. The duration of the flight of the balloons equalled 24 hours or more and the altitude reached was 30 km. Thus, the air balloons rose to the lower boundary of the zone of penetration of cosmic-ray particles. To carry out flight experiments with biological specimens, certain procedures were adopted for ensuring the necessary living conditions for various experimental animals (mice, etc.) during the flights with air balloons or in the capsule of a rocket (Lee, Henry, Ballinger, 1954; Simons, Parks, 1956; Kratochvill et al., 1959; Eugster et al., 1959). In these experiments the following were used: Neurospora, Drosophila, seeds of various plants (in particular, radish, barley), eggs and sperms of certain animals (e.g. long-horned grasshopper), eggs of crabs and shrimps, small pieces of tissue and skin taken from man or animals, cells in tissue cultures, as well as mice, guinea-pigs, hamsters, lower-order monkeys, etc. (Bushnell, 1958; Simons, 1954, 1957; Simons and Steinmetz, 1956; Eugster and Simons, 1960).

It is significant that due to the imperfection of the means used for ensuring the conditions of living, and for the return and rescue of the biological specimens, a large proportion of the experiments carried out during that time were unsuccessful: the biological specimens perished or could not be found (Bushnell, 1958). Therefore, in spite of the large number of experiments the material obtained was relatively meager and not always statistically reliable.

In experiments with air balloons for studying the traces of cosmic-ray particles, the method of photoemulsions is extensively used. This method allows investigation of the physical characteristics of cosmic radiation: the density of the particle flux, their charge, energy, the secondary processes that occur in the emulsion, etc. Furthermore, the technique of Eugster is frequently applied, according to which the effect of cosmic-ray particles is recorded simultaneously by means of photoemulsions and on various biological specimens (Eugster, 1957; Eugster et al., 1959, 1960; Yagoda, 1957, 1959; Yagoda and Smith, 1954; Schaefer, H. J., 1960).

In the enumerated American investigations most biological specimens and indices do not show any pronounced characteristics indicating the biological effect of cosmic radiation. The behavior and the higher nervous activity of the experimental monkeys, rats, mice and hamsters, which were observed for a long period after the flight, did not differ from the normal (Simons and Steinmetz, 1956; Harlow, Schrier, Simons, 1956). Post-flight histological investigations of the tissues of the animals, including the central nervous system (various methods of producing the preparations were used) also did not reveal any deviations from the normal (Campbell, 1954; Haymaker, 1956).

Calculations have shown that during a 24-hour flight, cosmic-ray particles should hit the crystalline lens of the eyes of mice several times. To study their influence on this organ newborn white mice, in particular, were used. In these, the transparency of the crystalline lens and other media of the eye were investigated after the flight by means of a slit lamp and microscopic methods. However, no trace of injury could be detected (Simons and Steinmetz, 1956; Cibis and Strughold, 1956).

Investigating 85 mice which had flown for several days in an air balloon (at an altitude of 24-32 km), Lebish et al. (1959) did not detect any appreciable changes as regards the average life span, the state of the nervous system and the higher nervous activity, particularly as regards formation of growths and various diseases (pneumonia). In these experiments no histological changes were observed in gonads or disturbances in the reproduction of progeny. According to the calculations of the authors, about 7350 particles with atomic number of $Z \geq 6$ penetrated into the group of mice investigated.

Pipkin and Sullivan (1959) placed 10,761 Drosophila larvae into the gondola of an air balloon flying according to the "Stratolab" plan (height about 24 km, flight duration 16 hours). Genetic investigations did not reveal any disturbances and induced mutations in the X-chromosomes of the experimental Drosophilae.

Slight genetic changes, which were not fully conclusive statistically, were detected in Neurospora (W. S. Stone, De Busk), eggs and sperma of grasshoppers (H. Walton). Experiments with eggs of crustaceans and tissue cultures (Simons and Parks, 1956; Bushnell, 1958) did not yield clear results either.

Experiments carried out on grains of barley revealed mutations: dwarfed and degenerate forms in the progeny of these grains. Photoemulsion layers for recording the tracks of particles were placed in the immediate vicinity of the grains. This enabled taking into consideration grains which were hit directly and frequently with cosmic-ray nuclei. Control grains (located on the ground) and experimental grains which were

not hit by cosmic-ray particles did not differ from the standard. However, grain hit directly by cosmic-ray particles yielded 30 to 40 percent less ears and grain, although the average weight of the grain yield (the weight of 1000 grains) did not change. Consequences of the experiment could be detected in the second, third and fourth crops. In some grains the color changed in subsequent crops. However, in these cases the number of ears and grains did not diminish in the yield. The ratio of the number of mutations to the number of sterilizations under the influence of cosmic radiation proved to be higher than under the effect of corresponding doses of X-rays.

Chase (1954), Chase and Post (1956), Simons and Steinmetz (1956), Simons (1954, 1957, 1958) and others observed a slight increase in the number of grey hairs in the $C_{57}$ line of black mice which were subjected to flight experiments. Simons and Steinmetz reported that in flight specimens the number of grey hairs was higher on the average by 10-11 than in the control specimens. In three mice Chase detected a group of grey hair which in his view served as evidence of passage of cosmic-ray particles. In one case all 12 grey hairs were located along a single line. Chase assumed that a particle track passed through there. In two other cases the grey hairs were distributed in such a way that it indicated collision of nuclei and formation of a "star" at this spot. Histological investigations of the skin of the mice carried out two months after the flight did not indicate any changes.

It is pointed out that all black mice have a small number of white hairs and their number increases with increasing age of the mice. Data on the greying of the hair in mice which have flown can hardly be considered as fully reliable from the statistical point of view. Chase, and particularly Simons and Steinmetz, also consider that their findings require further verification and confirmation.

Eugster (1955, 1957) sent up in air balloons and rockets specimens of human and rabbit skin which were specially treated with photoemulsions or special dyes (as mentioned above). After return the pieces of skin were carefully investigated and in some cases they were replanted on the donor. A pigmented spot was observed in a skin flap returned from flight, the color of which changed in the succeeding few months. The investigator explained the appearance of the spot in the skin flap as being due to the impingement of a heavy particle. Eugster considers that there can be no doubt of the injurious effect of cosmic radiation on small objects and the skin; it only remains to explain how local injury influences the entire body (Eugster, 1957).

In 1958-1960 ballistic rockets were launched in the US over distances of several thousand kilometers. These carried lower-order monkeys and mice on board. The flight duration was 15-20 min and during the

flight with monkeys an altitude of up to 500 km was reached. In individual cases the experiments were successful and the animals were rescued. Investigation of these did not reveal any aftereffects as regards radiation injury. In the flight of two monkeys (May 28, 1959) the recordings of the physiological functions and observation of the animals did not reveal any characteristics of the effects of cosmic radiation. However, the monkey Able died unexpectedly soon after the flight during a harmless operation for the removal of the implanted electrodes. The death of the animal was attributed to the unfavorable influence of the anesthetic, although out of 1000 cases (of which 700 were on monkeys) of such anesthesia this was the first fatality. A pathologic-anatomic autopsy did not reveal any symptoms of the effect of the flight (Graybiel et al., 1960; Biological Researches ..., 1959).

In addition to monkeys, other biological specimens were used during this flight:

1. Sea-urchin eggs and sperms which were intermixed during the flight. In one of the cylinders the eggs were fertilized immediately before takeoff, in the second during the period of maximum accelerations, in the third after return. The aim of these experiments was to elucidate the influence of cosmic rays, accelerations, vibrations and weightlessness on the sex cells, on the fertilization process and subsequent development of the embryo.

2. Human blood (the experiment was of practical importance for elucidating the possibility of transportation of blood by means of rockets).

3. Microbiological specimens (yeast cells, mold fungi, bacteria (intestinal bacilli)).

4. Plants: grains of corn and mustard, pieces of tissue of white and pink onions.

5. Drosophila

The investigations did not reveal conclusively injury to the biological specimens. It was pointed out in a preliminary communication that certain changes occurred in the eggs of the sea urchin: destruction of the eggs fertilized during the flight and particularly of nonfertilized ones; eggs that were fertilized prior to the flight were better preserved. In a later communication it was reported that this biological experiment was unsuccessful and did not provide a satisfactory basis for definite conclusions. In the same experiments, the induced radioactivity of flying objects and biological specimens was measured; the results were negative (Biological Investigations ..., 1959, Young, 1961).

From 1951 onwards regular flights were carried out with dogs in the Soviet Union in high altitude and then in ballistic rockets. Altitudes of 110, 220 and up to 450 km were reached. No aftereffects of cosmic radiation were detected in the experimental animals (Yazdovskiy et al., 1957; Bugrov et al., 1958; Galkin et al., 1958; Put' v kosmos (Way to Space), 1958: Stantsii v kosmose (Space Stations), 1960). However, the duration of the exposure of animals in these flights could not be long enough to judge reliably the biological effect of cosmic radiation.

In 1957 the historic flight of the dog "Layka" was achieved on the second artificial satellite. The physiological functions recorded during this flight did not reveal any influence of cosmic radiation of the animal (Chernov and Yakovlev, 1958). In spite of the long duration of the flight of Layka (seven days), the possibility of studying the biological effects of cosmic radiation was limited in view of the fact that the animal did not return to Earth and, therefore, could not be subjected to a thorough postflight investigation. "All these requirements were satisfied by the biological experiments which were carried out on the second and subsequent satellites."

## Biological Experiments on Spaceships - Earth Satellites

The second and subsequent spaceships (including the spaceships "Vostok-1" and "Vostok-2" which also contained some biological specimens) permitted the carrying out of extensive biological investigations. A specially devised program provided for the use of a variety of methods and procedures which unified ideas on obtaining comprehensive information on the biological effects of space flights, particularly of cosmic radiation (Sisakyan, 1960, 1961; Stantsii v kosmose (Space Stations), 1960, and others).

A multitude of physical investigations were carried out on spaceships with the aim of studying the composition and energy of cosmic radiation and the dynamics of the changes in these with time and space (in accordance with the orbit of the spacecraft). After returning from the flight, the induced activity of some materials which were inside the capsule was measured. The physical investigations carried out yielded new data on the radiation field in space along the orbit of the spacecraft.

The urine and blood of the dogs Belka, Strelka, Chernushka and Zvezdochka, as well as of some rats and mice (white and black) were subjected to comprehensive biochemical investigations before and after flight.

The aim of these investigations was to elucidate the effects of radiation and the factors that cause "stress reaction."

Immunologic investigations of microflora and the bactericidal properties of the skin and the phagocytic properties of the blood of the dogs are of great importance from the point of view of preparing for prolonged manned flights. The reaction of the peripheral vessels on the auricle of the dogs was also recorded. This method serves as a sensitive indicator of the effect of ionizing radiations (Yakovlev, 1958).

The animals were put under observation for a long time after the flight but no change has been observed in their general behavior and state. It is well known that the dog Strelka had two normal litters after the flight and they both developed satisfactorily. In black mice no greying of hair could be reliably observed. After the flight the experimental mice were subjected to histological investigations.

The blood and hematogenic organs of the animals were subjected to systematic study. A comprehensive series of cytogenetic investigations on the hematogenic apparatus, gonads and other organs were carried out.

A large number of microbiological and cytological specimens were exposed in the spaceships; this allowed study of the influence of cosmic factors not only on the level of highly organized complete organisms but also on the tissue, cell and subcell level. Use of microorganisms and of cells from the human body in tissue cultures enabled investigation of the degree of the influence of the flight on elementary genetic processes. As is well known, the second spaceship carried bacteria, bacteriophages, Hela cancer cells, DNA (deoxyribonucleic acid), small pieces of human and rabbit skin (reimplanted on the donors after the return of the spaceship) and other specimens.

The results of the experiment with chlorella are of considerable interest since chlorella as well as some other plants and animals will obviously play a major role in ensuring the necessary conditions for the survival of man in future space flights.

The genetic investigations on spaceships were carried out on a large scale. The materials used included: Drosophilae, actinomyces which produce antibiotics, flowering spiderwort, peas, maize, wheat, onion and nigella. The importance of studying the genetic effects of space flights is obvious. At present it is important to carry out a further analysis of the effects of individual flight factors (weightlessness, radiation, vibrations, accelerations) and their interaction as well as the influence on cell structures.

During preparation for experiments in spaceships a great deal of work was carried out on the experimental methods to be adopted.

The following were developed:

a) methods of exposing biological material during space flights;

b) means for ensuring the conditions for survival of the biological specimens (cages for mice with appliances for feeding and water supply, thermostats for "bioelements", perspex containers for spiderwort, Drosophilae, actinomyces, etc.);

c) means for obtaining information on the performance of the "bioelements" during flight as well as methods of subsequent evaluation and analysis of the material, etc.

In experiments on the third, fourth and fifth spaceships, the complement of experimental biological specimens was considerably extended. Additionally, guinea-pigs, gray mice, seeds of buckwheat, broad beans, and onion seed sprouts in various stages of development were included.

It is of great importance to supplement the microbiological specimens with lysogenic strains, since lysogenesis is one of the most sensitive tests regarding the effect of radiation. In addition to cultures of epithelial cancer cells of man (Hela), pulmonary epithelial tissue, cells of human embryonic and amniotic tissue, fibroblasts and bone marrow of a rabbit were used. To study the initial stages of development of a fertilized egg, frog eggs and sperms were used which were automatically intermixed at a given instant in accordance with a preestablished program. A number of microbiological specimens were used repeatedly (to investigate the cumulative effect of the flight factors): culture of intestinal bacillus KK-12, bacteriophages type T-2, Hela cancer cells, etc.

For studying the biological effects of cosmic radiation, the following biological specimens were used on the third, fourth and fifth spaceships:

a) a collection of enzymes (pepsin, trypsin, alkaline phosphataze, catalase, peroxidase, ribonucleaze);

b) homogenate from wheat germs;

c) preparation of cell nuclei (wheat);

d) tobacco mosaic virus of various strains (ordinary cyphomandric and plantaginaceous) in aqueous solution and ammonium sulfate;

e) influenza virus (on various tissue cultures).

Some Results of the Experiments on Spaceships

Dosimetric instruments installed in the capsule of the second spaceship have shown that the total absorbed dose (Savenko, Pisarenko, Shavrin, 1961; Savenko, Pisarenko, Shavrin, Papkov, 1962; Keprim-Markus, Kovalev, Uspenskiy, 1962) of cosmic radiation inside the ship equalled about 10 mrad. No induced radioactivity could be detected in the specimens which were inside the spaceship (Matveyev and Sokolov, 1962).

Biochemical investigations of the blood and urine of Belka and Strelka after their 24-hour flight on the second spaceship have revealed a transient and moderately pronounced "stress" type reaction which consisted in a short duration increase of $\alpha_2$-globulins and serum mucoids

(2-6 days after returning). Then all the indices reverted to normal. In the dogs (Chernushka and Zvezdochka) who performed a short duration (a single orbit) flight on the fourth and fifth spaceships, no disturbance in their metabolism could be detected.

The effect of accelerations (6-9 g, 1-5 min) or of vibrations (70 cps, with an amplitude of 0.4 mm for durations of 5-12 min) under laboratory conditions brought about an increase of the nonspecific cholinesterase activity and a drop in the concentration of serum mucoids in the blood, which indicates activation of the function of the liver and possibly of the reticulo-endothelial system. Intensive investigations, training and testing of the dogs led in some cases to dystrophic states, characterized by a steady increase of concentrations of serum mucoids and globulin in the blood and reduction of the total quantity of proteins at the expense of albumins. Dogs who have flown several times in high-altitude rockets revealed certain peculiarities in their reactions to the effects of accelerations, vibrations and intensive work.

A single flight into space did not bring about irreversible changes in metabolism. The dynamics of changes in the investigated indices after the flight is more characteristic of stress reactions than of radiation injuries (Gyurdzhian, Demin, Korneyeva, L'vova, Tutochkina, Uspenskaya, Fedorova, 1961).

Investigations of factors of natural immunity after space flights of dogs (quantity of microbes on the surface of epidermis, bactericidal properties of skin sections, phagocytic and bactericidal properties of blood) showed a wavelike change in the immunological reactivity with predominance of activation phases. Certain variations in these parameters were also observed in laboratory experiments in which the effect of accelerations and vibrations was investigated, as well as in dogs which had previously flown several times in high-altitude rockets (Alekseyeva, 1962).

The state of the peripheral vessels of the dog's ear (arterial and venous pressure, arterial and venous tone, speed of blood circulation) investigated by means of a universal pressure gauge during and after the flight did not reveal any appreciable shifts in hemodynamics (Yakovlev, 1962).

In two rats which flew on the second spaceship, and seven control rats, the conditioned reflex activity (standardized six positive and one differentiated motor-alimentary conditioned reflexes from the auditory and visual analyzers) was studied. The higher nervous activity of the rats which participated in the flight did not reveal any changes or features as compared to the control rats (Luk'yanova, 1962).

Microbiological and cytological investigations carried out in experiments on spaceships were aimed at solving the following two basic biological problems:

1) study of the conditions of life in cosmic space;

2) study of the genetic changes caused by the effect of various factors in space.

The experiments on spaceships did not reveal any appreciable influence of flight conditions on the microbiological and cytological specimens. Small pieces of skin reimplanted on the donors behaved in a similar manner to control pieces which had not participated in the flight. Cells in tissue cultures retained their viability and ability to multiply; bacteria and phages retained their functional and morphological properties; experimental samples of DNA did not differ from the control samples; only lysogenic bacteria revealed initial changes in one of the experiments and this will require further careful study (Zhukov-Verezhnikov et al., 1961). The culture of the water plant chlorella also fully retained its viability and did not show any irreversible changes in the basic physiological processes: Photosynthesis, growth, development and multiplication (Semenenko, Vladimirova, 1962).

Thus, the described experiments did not reveal any injurious effects of space flight; this applies not only to a highly organized multicell organism as a whole but even to single cells.

Cytogenetic investigations of the bone marrow and spleen of mice which flew on the second spaceship revealed certain chromosome disturbances as well as phase depression and intensification of hematopoeisis. The above mentioned chromosome disturbances have a number of qualitative features: adhesion of the chromosomes instead of the usually observed fragmentation; chromosome disturbances are observed a long time after the flight (Arsen'yeva, Antipov et al., 1961).

In two strains of Drosophilae a slight increase in the frequency of the dominant and sex-linked recessive lethal mutations were observed. In the spermatides the frequency of mutations is higher than in sperms. The majority of the mutations are of the punctatic type. Still higher is the percentage of mutations in strains with a high spontaneous mutability (Glembotskiy, Abeleva et al., 1961; Parfenov, 1961).

To elucidate the mechanism of occurrence of such disturbances and to study separately the influence of various flight factors, laboratory experiments were carried out in which biological specimens were vibrated. The vibrations produced cytogenetic changes in the organs of mice and an increase in the frequency of mutation in Drosophilae which are commensurate with the influence of space flight. This indicates that the influence of the mechanical factors of flight have to be carefully studied (Arsen'yeva, Antipov et al., 1961; Glembotskiy, Abeleva et al., 1961; Parfenov, 1961; Dubinin, 1961).

Actinomyces also showed a definite reaction to flight conditions. Radiation-sensitive strains showed a decrease while radiation-insensitive strains showed an increase of their viability. However, in all the strains the surviving spores revealed a pronounced stimulation of growth, formation of thick hyphae and a large mass of mycelium (Glembotskiy, Prokof'yeva-Bel'govskaya et al., 1961).

In cytological analysis of sprouts from seeds of agricultural plants, slightly pronounced changes were observed in the percentage of chromosome rearrangements as well as changes in the mitotic index (rate of cell division). Thereby, in radiation-resistant strains, the tempo of cell division increased preferentially and the percentage of chromosome rearrangements changed relatively little. In radiation-sensitive strains the percentage of chromosome rearrangements increased and the tempo of cell division decreased somewhat (Glembotskiy, Prokof'yeva-Bel'govskaya et al., 1961).

Examination of dry seeds of radiation-sensitive Allium fistulosum (spring onions) and of radiation-insensitive Nigella damascena (Love-in-the-mist) revealed complete absence of any influence of the flight conditions on the frequency of chromosome rearrangements and a clear positive influence on the sprouting and germination of seeds. This phenomenon is explained as not being due to the effect of radiation but to other effects, possibly mechanical factors in flight (Sidorov, Sokolov, 1961).

The general result of the biological investigations associated with experiments on spaceships is the conclusion that flight of man under conditions pertaining in the second and subsequent spaceships does not present any danger from the radiological point of view.

The scientific importance of individual weakly pronounced shifts observed by means of genetic, cytogenetic and pathohistological methods (Petrukhin, 1961) consist only in the fact that they draw our attention to problems which require further more detailed study (particularly the influence of mechanical factors and the combination of various flight factors), and facilitate the extremely difficult problem of selecting the most sensitive and adequate methods of investigation. The quantitatively expressed genetic shift, revealed by means of highly sensitive and subtle methods, is very small. It does not present any somatic or genetic danger to the cosmonaut.

Thus, biological experiments on spaceships and satellites have opened up the way for manned flight into space. They provided the necessary medicobiological basis and the biological experiments preceding the flights of Yuri Alekseyevich Gagarin and German Stepanovich Titov on the spaceships "Vostok-1" and "Vostok-2". As is well known, very detailed and thorough medical examinations of the cosmonauts before and after the flights have shown that ionizing radiations had no influence on them.

Biological investigations carried out on the American satellites "Discoverer-17" and "Discoverer-18" confirmed the basic results of the experiments carried out on Soviet satellites; the flight of the satellite "Discoverer-17" (November, 1960) coincided with an intensive sun flare. During the 50 hours of its flight (31 orbits), the biological specimens were subjected to irradiation with a total dose of about 30-35 rad (through the entire flight time hits by $10^8$ protons and $10^6$ heavy nuclei per 1 $cm^2$ of the surface of the specimens were registered).

In these experiments the following biological specimens were used: cultures of human tissues (cells of the synovial membrane and cells of conjunctiva), preparations of human and animal blood on filter paper, bacterial spores and chlorella cultures.

According to preliminary data, no important signs of injury were detected in the biological specimens which were on the satellites "Discoverer-17" and "Discoverer-18". The cultures of tissues which were on the satellites, as well as the controls, first passed through a short phase of degeneration (which was caused by the exhaustion of the nutrient medium) and then all their properties were restored. Clostridium sporogenes which were in the satellites proved somewhat more resistant to the destructive effect of a hot solution of caramelized sugar than the control specimens (Bulban, 1961).

Investigations carried out in the USA on Neurospora, which were lifted by means of rockets to an altitude of 1180 miles, revealed important genetic and physiological changes in these as compared to control specimens (De Busk, 1961).

To ensure the safety of the astronauts against radiation in the satellites "Vostok", in addition to solving technical problems of protecting the capsule of the spaceship against radiation, it was of great importance to make a correct choice as regards the timing of the flight as well as to monitor continuously the state of the radiation field in the space surrounding the Earth, to watch for possible sunflares. This was particularly important during the flight of G. S. Titov which lasted 25 hours. In the period preceding the launching and during the entire flight a special program was in operation for observing the activity of the Sun and for direct measurement of the intensity of cosmic radiation in the upper layers of the atmosphere. A wide network of astronomical establishments observed various aspects of the activity of the Sun. The results of astronomical observations were synthesized and served as a basis for forecasting the probability of an increase in the activity of the Sun and occurrence of flares. Simultaneously the intensity of cosmic rays in the stratosphere was measured by means of sounding balloons at various latitudes of the Soviet Union. All this information enabled the flight controllers to make a decision on the time when the launch should be made and on the subsequent programming. Directly prior to the launch and during the entire flight time the activity of the Sun, as well as the intensity of cosmic radiation in the stratosphere, were within specified limits. From the radiation point of view, the flight conditions were favorable (Pravda, September 8, 1961).

So far no convincing experimental data have been obtained which prove the injurious biological effects of cosmic radiation. However, it would be premature to make final conclusions on the biological effects of cosmic radiation at present. Future flights will penetrate to greater depths into space and will have a continuously increasing duration. The orbits of the satellites "Vostok-1" and "Vostok-2" were favorable from the radiation point of view (they were below the periterrestrial radiation belts) and the maximum flight duration was relatively short (only days). Furthermore, the radiation field surrounding the Earth is continuously subjected to enormous fluctuations in time and space both as regards intensity and composition. Therefore, the results obtained in these experiments are only valid for the given actual flight conditions. In particular, solar flares, which are extremely difficult to predict, and the resulting changes in the radiation field in the direct neighborhood of the Earth, give rise to great anxiety (Charakhch'yan et al., 1961; Gorchakov, Bazilevskaya, 1961).

The biological experiments so far carried out provide the groundwork to future very important research. Further systematic and thorough investigations, as well as improvements in the methods and indices used, will be required. It is necessary to obtain detailed information on various zones of cosmic space as well as changes with time of cosmic radiation.

Biology and medicine are faced with major and difficult, but interesting and gratifying, tasks relating to the study of the biological effects of cosmic radiation. This study represents one of the most important foundations for mastering outer space.

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## SUMMARY

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Nearly 300 references, both Russian and Western, relating to the physical and medical aspects of space flight are quoted and briefly reviewed.

Fundamentals of cosmic radiation, radiation belts around the Earth and interreaction of various radiations with the environment are given. The effects of ionizing radiations on tissues and the principles of protection against cosmic radiations are discussed. The importance of solar activity forecasts is discussed. The results of experiments exposing biological material to cosmic radiation are also discussed. There are 21 figures and 6 tables.

Author

Translator's note: This is an original abstract and not a copy of the English "summary" printed with the article.

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# PROBLEMS OF BIOLOGICAL TELEMETRY

Parin, V.V., Bayevskiy, R.M. and Gazenko, O.G.

The remote recording of physiological functions by radiotelemetry finds increasing application in medicine and biology. At present, radiotelemetry methods are used in athletics, aviation and cosmic medicine. Equipment recording the pulse, breathing, biocurrents of the muscles and other parameters of sportsmen during training and competitions have been developed (Rozenbalt, 1959; Unzhin, 1959; Sarychev, 1958). An instrument for studying pilots in flight was constructed by Glatt et al. (1953), Barr (1954), A.N. Prutskiy (1956), G.V. Samoylev et al (1960). The first experiments in the use of radiotelemetry in cosmic medicine are credited to Henry et al (1952), who recorded physiological events in monkeys during vertical launching of rockets. A large volume of radiotelemetry investigations on animals was assembled during the period of the launching of the second Soviet artificial Earth satellite (Chernov, Yakovlev, 1958). During this, information on the state of living organisms under conditions of orbital flight was first obtained.

A special branch of radiotelemetry concerned with the transmission of biological data is referred to as "biological telemetry." Biotelemetry is the fundamental research tool and monitoring method of cosmic medicine. Any measurements in cosmic flight depend on the use of radio channels. The presence of a radio link between the object under observation and the recording instrument affects the methods and the technique of medical-biological investigations. This work is concerned with the use of biotelemetry in space medicine. All these problems are neither purely technical nor purely medical. Their solution can be achieved only as a result of creative cooperation between physicians and engineers.

Unlike laboratory experiments, where physiological data can be recorded in any quantity and practically in accordance with any given program, during a cosmic flight the volume of information received is strictly limited and the recording sessions depend on the parameters of the orbit. The orbit of an artificial satellite around the Earth is such that communications between the receiving stations and the radiotelemetering station aboard the satellite are intermittent. In order to obtain a sufficient quantity of information and to ensure its continuity, a special network of receiving stations is established. However, in view of the motion of the artificial satellite in the orbit and the rotation of the Earth, it is impossible to obtain equal quantities of information during each orbit. During various time intervals of up to several hours, it is difficult to achieve direct radiotelemetry communication. During such periods, only a limited quantity of operative information which is obtained by means of special radio devices is available. One of the difficult problems arising in this connection is the programming of

telemetering during cosmic flight. It is necessary to obtain the maximum volume of information on various sections of flight through different telemeter and radio systems.

The telemetering program is prepared by analyzing a given experimental flight and the possibility of using various investigation methods during flight. At present, the problem regarding the volume of medical and biological information, which is transmitted from cosmic ships and satellites to Earth, is widely discussed in specialized literature. Table 1 lists the parameters included in the investigation programs proposed by various authors.

The simplest metering program, described by Kolcum, was designed for studying the first flights of the project "Mercury." This program is essentially designed for medical examination of an astronaut in flight. Other programs are of the research type concerned not only with providing the necessary minimum of measurements but also with obtaining a wide range of information permitting analysis of the influence of various flight factors and their effect on the individual systems and organs of a living organism.

When choosing the methods of investigation in cosmic flight and arranging a telemetry program physiologists should take into account specific conditions of the experiment and, in particular, the design features of the medical equipment on board and the problem of transmission of medical-biological information by radiotelemetry. The biotelemetry instruments on board comprise sensors-transducers, amplifying-converter units and radio-transmitting equipment. It is generally required that this equipment should be light in weight and small in size, highly economical and reliable, and stable during vibrations, overloads and extreme temperatures. Sensor-transducers designed for operation during flight should be miniaturized, be able to operate without distortion over long periods of time and should not interfere with the work of the astronaut. All this necessitates the development of special equipment which should meet the above requirements.

Special medical-investigation equipment for transmission of information over a number of radiotelemetering channels was designed in the USSR for carrying out physiological investigations on animals. This equipment was used in experimental flights on the second and third Soviet satellites and enabled the collection of a large volume of medical and biological information (Table 2).

Data obtained during the experimental flights made it possible to select physiological methods used for medical monitoring during the first manned flight. Special equipment was designed for this flight, by means of which it was possible to take electrocardiogram leads in several regions of the body, as well as pneumograms. The equipment was first

Table 1. Telemetering Program in Space Flight, According to Data of Various Authors

| Physiological parameters | Helvey (1959) | Traite (1959) | Powell (1959) | Kolcum (1960) | Miller (1961) |
|---|---|---|---|---|---|
| Electrocardiogram (pulse) | + | + | + | + | + |
| Heart tonus | + | + | + | - | + |
| Rate of propagation of pulse wave | - | - | + | - | - |
| Electroencephalogram | + | + | + | - | + |
| Electromyogram | + | - | + | - | - |
| Pneumogram | + | + | + | + | + |
| Arterial pressure | + | + | + | - | - |
| Dermogalvanic reaction | + | + | - | - | - |
| Conditioned motor reflexes | + | - | + | - | - |
| Thermometry of skin and body | + | + | + | + | + |
| Activity of gastro-intestinal tract | + | - | - | - | - |
| Degree of blood saturation with oxygen | - | - | + | - | - |

Table 2. Telemetering Program on Second and Third Soviet Space Satellite Ships

| Object | Animal | Methods of investigation |
|---|---|---|
| Second Soviet artificial satellite-ship | Belka<br>Strelka | Electrocardiography, Phonocardiography, Arterial oscillography, Pneumography, Actography, Thermometry |
| Third Soviet artificial satellite-ship | Pchelka<br>Mushka | Electrocardiography, Phonocardiography, Seismocardiography, Pneumography, Actography, Thermometry, Electromyography |

tested during flight experiments with the dogs Chernushka and Zvezdochka. Here, apart from the electrocardiograms and pneumograms, sphygmogram of the carotid artery was recorded, which supplemented the investigation programs of the second and third cosmic satellite ships. In this way, a vast program of medical and biological investigations was realized in the Soviet Union under conditions of space flight using altogether ten different physiological methods (including studying the behavior of animals by means of a television link). Figure 1 shows samples of radio-telemetry records obtained during flight experiments. These physiological

investigations were only the first steps of biological telemetry in cosmic medicine. They can be regarded as a forerunner of the future possibilities of radiotelemetry. Introduction of various new methods is envisaged as well as improvement of the existing methods.

One of the important problems of modern biotelemetry is the matching of the volume of the information transmitted by radio channels with a channel capacity and the increase of the quantity of information transmitted along an individual channel. Here, we face the necessity of using the information theory in the problems of physiology. Determination of the quantity of information to be transmitted by a radiotelemetering channel is one of the problems. The quantitative evaluation of the information contained, for example, in an electrocardiogram is never considered by physicians. What they are looking for are differences from the generally accepted standard. The more frequent are such deviations, the more information a physician receives. Sometimes, a great deal of information is supplied to a physician by obtaining a standard curve from a sick patient. An electrocardiogram is studied in quite a different manner by an engineer who pays no particular attention to the form of the trace, its amplitude or time relationship. For an engineer, an electrocardiogram is a set of various signals which are transmitted by a radio channel at a given rate. The transmission of each signal implies that a given signal was chosen from a set of all possible signals. Consequently, the "information in the abstract sense is simply the choice of a given element from a set of possible elements" (Poletayev, 1958).

For determining the quantity of information it is necessary to know the overall number of possible signals under given conditions and the probability of their appearance. For simplicity, we shall consider all signals as being equally probable. Selection from two equally probable states is the unit of measurement of the quantity of information. The information is measured in binary digits per second in accordance with the formula

$$H = n \cdot \log_2 N$$

where H is the quantity of information in binary digits per second, n is the rate of transmission and N is the number of possible equally probable states.

The rate of transmission of information over a radiotelemetering channel depends on the maximum frequency of the process under investigation. Thus, for transmitting an electrocardiogram without distortion, not less than 100 signals per second are required (Figure 2). For transmitting a pneumogram it is sufficient to send 5 signals per second. The frequency of the signals to be transmitted determines the bandwidth of a radiotelemetering channel. The number of possible values of signals (probable states) can be calculated from the maximum level of the

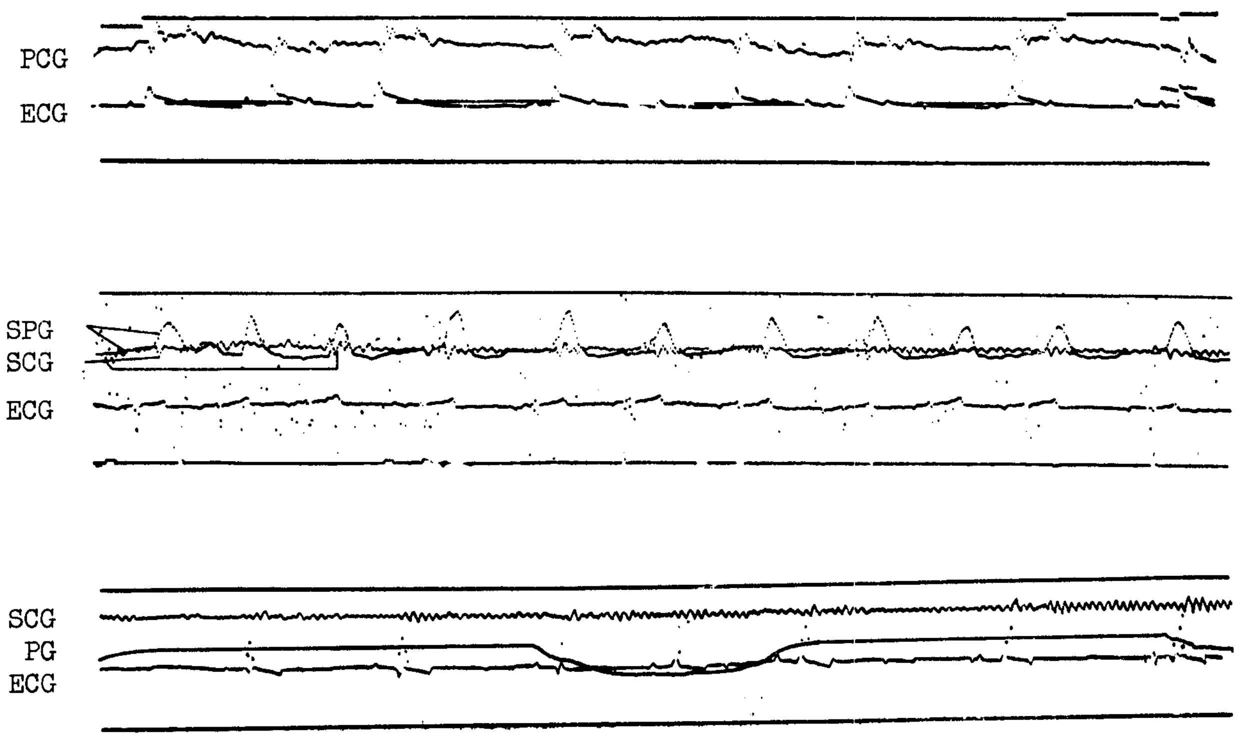


Figure 1. Samples of Radiotelemetric Recordings, Received During Medical and Biological Experiments on Soviet Spaceships With Animals on Board. 1) PCG - phonocardiogram; 2) ECG - electrocardiogram; 3) SPG - sphygmogram; 4) SCG - seismocardiogram; 5) PG - pneumogram. The upper ECG was recorded by an amplifier with a detector and an integrator connected to its output.

information to be transmitted and the required accuracy of transmission. Thus, for example, if it is necessary to transmit an electrocardiogram in the form of electric voltage signals, the maximum level is determined by the highest peaks. Thus, with a maximum amplitude of QRS of 5 V, and an accuracy of 5% (0.25 V), 20 different amplitudes can appear at the input of the radio channel, i.e. 0.25, 0.50, 0.75 V and so on up to 5 V (Figure 2). If the number of possible signals and the required transmission accuracy are known, the quantity of information contained in an electrocardiogram can be determined from the above formula. This corresponds to about 400 binary digits per second. This figure is of no practical interest to a physician but it is important for an engineer when designing radiotelemetering channels for the transmission of physiological data. Each radiotelemetering channel is designed for transmission of a predetermined quantity of information. The channel capacity is also measured in binary digits per second. The efficiency of a radio channel is given by the ratio of the quantity of information which can additionally be accommodated in a channel without distortion to the channel capacity. The lower the channel capacity, the higher the efficiency. For channels having a capacity of 10 or less binary digits per second, the efficiency is close to unity, while for channels of larger capacity this varies from 0.05 to 0.75. The capacity of a radiotelemetering channel is given by the formula:

$$C = W \cdot \log_2 (1 + P/N)$$

where C is the capacity of the channel in binary digits per second, W is the frequency bandwidth in c.p.s., P is the average power of the transmitter and N is the average noise power. Communications engineers consider that a reduction in the bandwidth of the channel with a simultaneous increase in the transmitter power or vice versa, does not reduce the channel capacity. However, during transmission of physiological information this is not so. If during the transmission of an electrocardiogram the rate is reduced threefold, down to ~30 signals per second, the recording of a QRS waveform with a duration of 0.05 - 0.08 sec should be accomplished by two to three signals, which is obviously insufficient. Table 3 shows the oriented data based on the quantity of information contained in certain physiological parameters and the optimum channel capacity for their transmission.

As is seen from the table, channels of different capacity and bandwidth are required for the transmission of physiological information. For a number of parameters channel capacities of 500 binary digits per second are necessary, while for some parameters the channel capacity can be less than 10 binary digits per second.

In order to reduce drastically the channel capacity and the number of channels, it is possible to use methods of information-coding. The term "coding" has wide use in radiotelemetry. It implies converting one type of signal into another, which is more suitable for transmitting

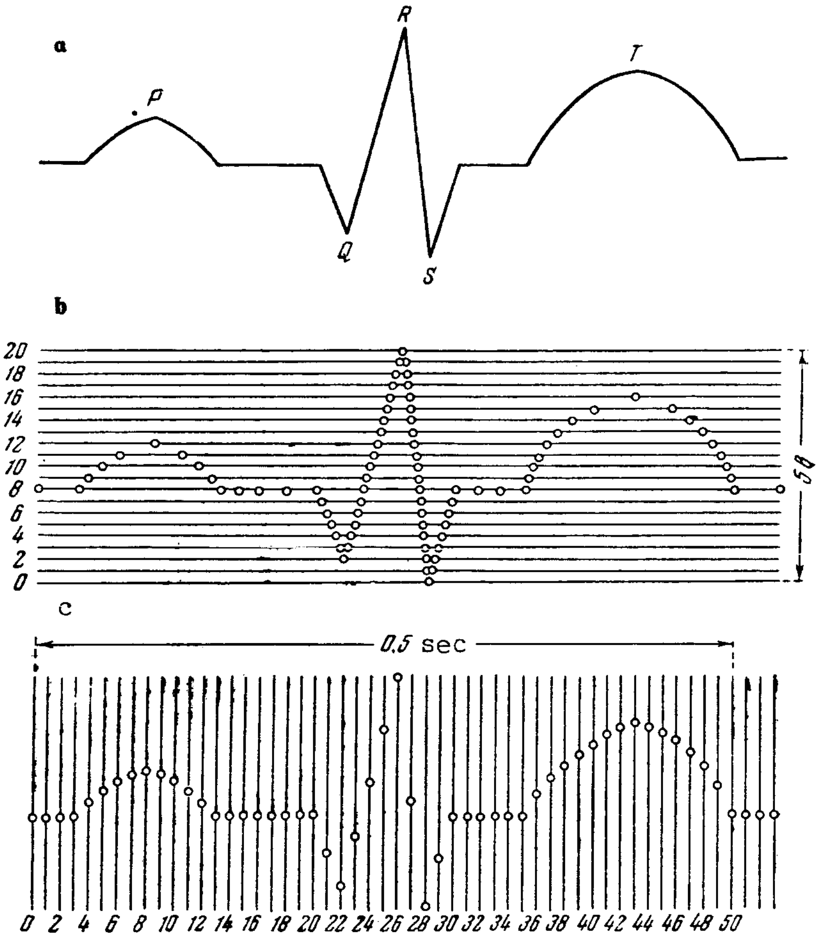


Figure 2. Calculation of Capacity of a Telemetry Channel for Transmission of an Electrocardiogram. a - electrocardiogram (usual recording); b - quantization of electrocardiogram (in amplitude); c - electrocardiogram at transmission speed of 100 per second.

through a given channel. Thus, the conversion of audio-oscillations into electrical signals by means of a microphone is a simple example of coding. In the wider sense, any process of information-conversion can be referred to as "coding": transmission of letters by Morse code; the conversion of a visible image into a series of electrical signals by means of television equipment; recording of the heart sounds as a waveform (phonocardiogram). The process of interpreting an electrocardiogram by a cardiologist can be regarded as a coding process converting the signals which are understood only by him (amplitude, time and morphological peculiarities of the waveform) into numerical and verbal concepts, connected with various ailments which are better understood by a

Table 3. Quantity of Physiological Information and Capacity of Radiotelemetry Channels Required for its Transmission (Maximum level - 5v)

| Physiological parameters | Required accuracy of reading, v | Number of possible values | Required speed of transmission per second | Quantity of information | Channel capacity |
|---|---|---|---|---|---|
| | | | | Binary units per second | |
| Electrocardiogram | 0.25 | 20 | 100 | 400 | 500 |
| Electroencephalogram | 0.50 | 10 | 200 | 600 | 800 |
| Electromyogram | 0.50 | 10 | 1000 | 3000 | 4000 |
| Pneumogram | 1.00 | 5 | 5 | 10 | 10 |
| Actogram | 0.50 | 10 | 20 | 60 | 75 |
| Thermogram | 0.10 | 50 | 0.05 | 0.25 | 0.25 |

large number of people. The automatic processing of the data performed by some modern diagnostic instruments such as determination of the frequency-amplitude contents of electro-encephalograms by means of an electronic integrator-analyzer is also a coding process.

Use of the methods of coding in transmission of physiological data by radio channels is of importance, not only in the conversion of the information into a form more suitable for transmission through a given channel but also as regards the possibility of automatic processing of scientific data. The latter is particularly important in the design of the equipment for medical monitoring, where the evaluation of information should be carried out continuously. Thus, in order to obtain operational data on the pulse-frequency in the equipment installed in an artificial Earth satellite, special equipment (cardiophone) was installed which converted the electrocardiogram into electrical signals having a duration of several hundreds of milliseconds, which corresponded to the pulse-frequency (Figure 3a). These signals were continuously transmitted by short-wave transmitters on board in the form of audio-messages, which ensured a dynamic medical monitoring of the pulse frequency. Transmission of the pulse signals by means of the cardiophone represents the simplest example of coding of physiological information. At present, various new systems of coded medical monitoring are available. One of these systems (a computing-coding unit, CU) produces continuous information relating to the pulse rate, breathing rate and motor activity (Figure 3b). Computing and integrating circuits, pulse-width and pulse amplitude modulation (PWM or PAM) are used in SCB. The next step in the development of methods of cosmic medicine is the design of systems of automatic medical monitoring, where an overall picture of the observations recording the state of a human being or an animal in flight will be transmitted in the form of a single signal. A proposal for such a system based on a binary code was published by McLenan (1959).

For obtaining continuous medical monitoring under conditions of cosmic flight, it is possible to use computing techniques. The production of generalized data measuring the state of several parameters can also be referred to as "information-coding."

Such coding, based on miniature electronic digital computing devices and using a suitable algorithm, will make it possible to transmit information to Earth by means of channels of very low capacity, to include this information in the control system (e.g. for automatic switching of safety devices in the case of a rapid deterioration in the state of an astronaut) and to obtain continuous information on the state of the spacecraft (Figure 3c).

Carbery (1961) proposes to use digital computers for fast processing of radiotelemetry information on Earth. The systems of automatic medical monitoring are particularly important for long-distance and prolonged

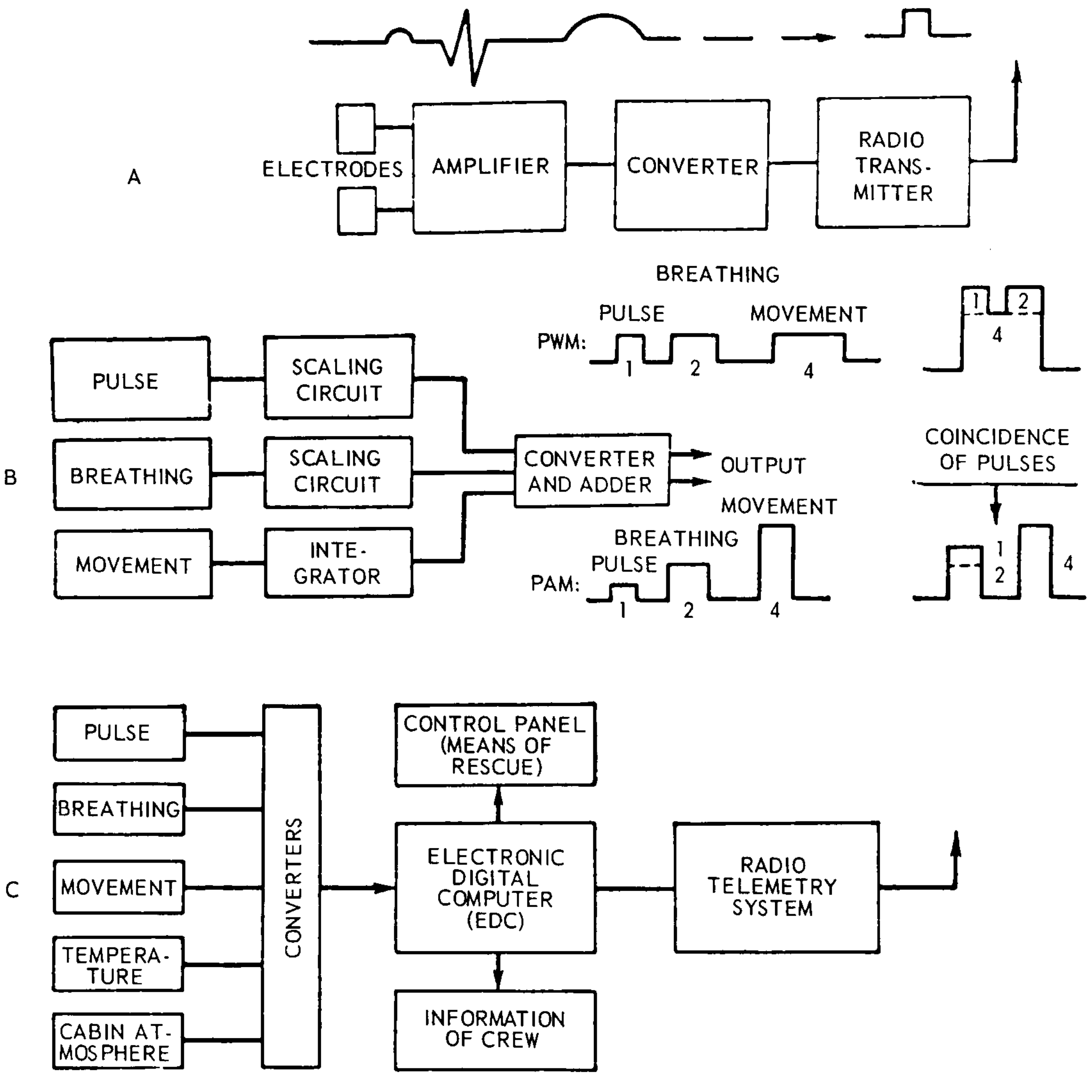


Figure 3. Methods of Coding Physiological Data. a - Transmission of pulse by means of cardiophone; b - Single-channel coding of three parameters; c - coding of information by means of EDC.

cosmic flights. The value of the information under these conditions increases rapidly since the quantity of the information transmitted to Earth is proportional to the weight of the ship (McLenan, 1959).

Increase in the duration of cosmic flights poses a new problem for biological telemetry; namely, ensuring medical monitoring of astronauts and investigation of animals under conditions of weightlessness. Instead of using a wire link between the object under investigation and the radiotelemetry system, a radio link should be used, i.e. the object under

investigation should carry a miniature radio transmitter which transmits information to a receiving device situated inside the cabin. The systems using relaying of the data from the object to the radiotelemetering equipment on board are called "the systems of mini-radiotelemetry." In cosmic medicine mini-radiotelemetering systems are employed mainly for ensuring a continuous medical monitoring or for biological investigation. The term "biological investigation" means the use of animals in order to assess the conditions which are dangerous to life. Figure 4 shows a block diagram of a relaying biotelemetry system; a diagram of laboratory recording equipment for physiological events and a normal biotelemetry system on board is also shown in the figure for the purpose of comparison.

New achievements in cosmic medicine are closely connected with the development of biological telemetry, and the development of reliable medical monitoring and medical-investigation systems. The most complete exploitation of radiotelemetry channels viz., increase in the information density, increase in the information content of the signals transmitted to Earth - these are the basic problems of biological telemetry and cosmic physiology. In connection with these problems it is most important to obtain efficient methods for coding medical and biological information and to employ computing techniques for logic analysis of the data obtained. Future research-type biotelemetry systems should effect automatic selection of the most "weighty" information, its rational coding and programming of the processing conditions and the information-transmission to Earth. Here, the problems of biotelemetry are closely linked with those of cybernetics, whose achievements should be widely taken into account in cosmic medicine.

As the basic method of medical investigation and medical monitoring, biotelemetry should be one of the most important factors in scientific-research works in the field of cosmic medicine. Successful solution of the problems confronting biotelemetry is only possible in close creative collaboration of physicians and engineers. The work in the field of biotelemetry compels a physician or biologist to consider the flight experiments not only from the point of view of the correctness of formulation of the scientific problems and choice of methods for its solution but also from the point of view of the possibility of obtaining scientific data, i.e. in effect, obtaining an answer to the problems. In fact, without knowledge of the problems connected with transmission of information to Earth and the methods of coding, it is neither possible to plan experimental flights nor to evaluate objectively the information received.

The use of radiotelemetering channels for the transmission of medical-biological information uncovers a wide field for an engineer. However, the work of an engineer in the field of biotelemetry is impossible without knowledge of the basic concepts of cosmic physiology and without understanding of the problems confronting cosmic medicine.

In this way, biotelemetry is today one of those branches of science where further progress depends on the mutual understanding between representatives of medicine and engineering and on their creative initiative.

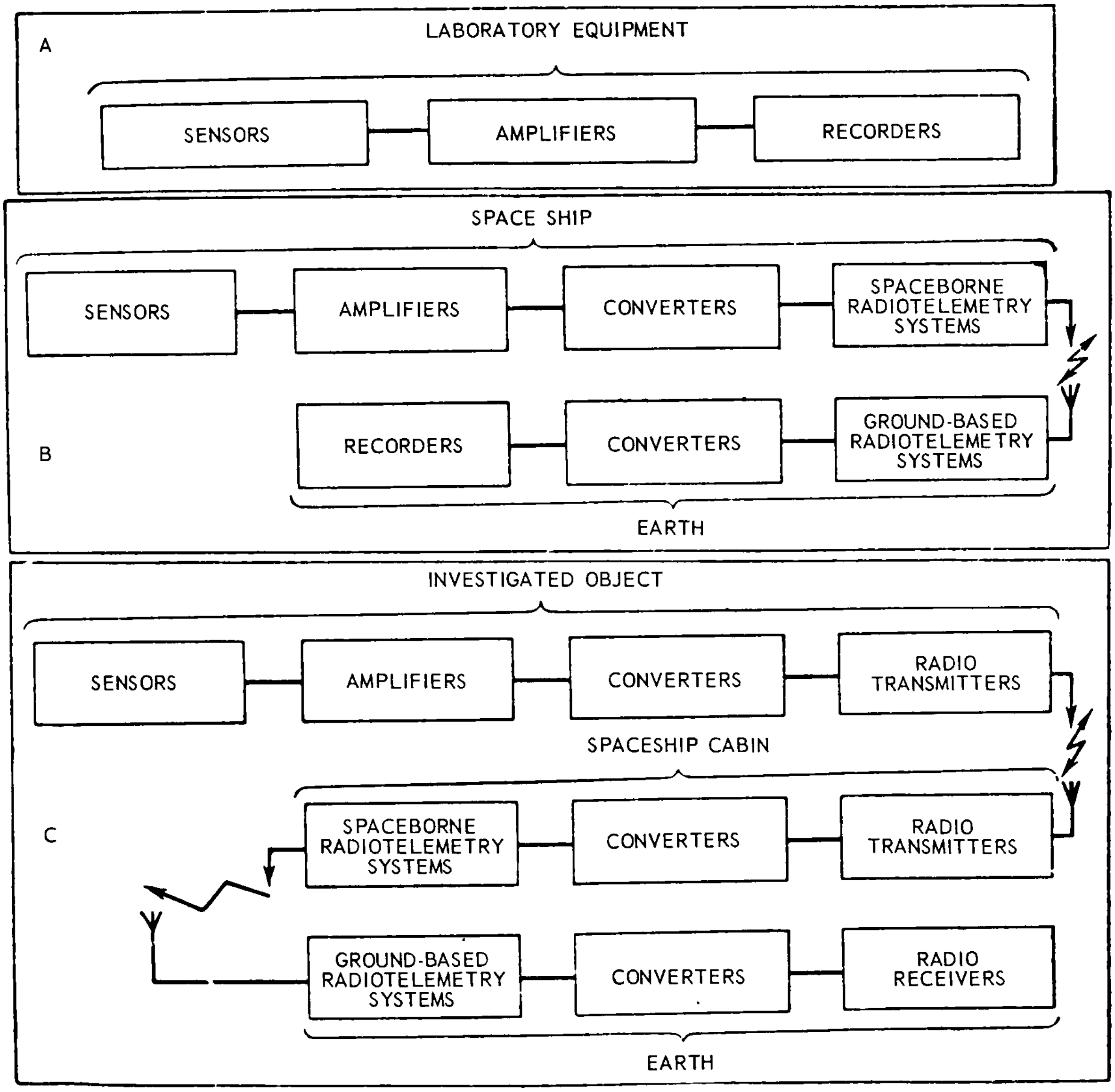


Figure 4. Types of Radiotelemetry Systems (RTS)

SUMMARY

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Registration of various physiological functions in the unique conditions existing during space flight is discussed. Particular stress is given to the need for suitable programming of the information conveyed by the various sensors during the space flight. The main program of the intelligence collected during space flight included: electrocardiogram, cardiac tone, pulse wave propagation, electroencephalogram, electromyogram, pneumogram, arterial pressure, galvanic skin reaction, various motor reflexes, skin and body temperatures, activity of gastro-intestinal tract and oxygen saturation of the blood.

The methods used during the second and third satellite flights carrying dogs included: electrocardiography, phonocardiography, arterial oscillography, pneumography, actography, thermometry, electromyography and seismocardiography. Samples of the radiotelemetric recordings of the tracks from the above measurements are given. Problems connected with the transmission and channelling of the intelligence are also discussed and it is concluded that the reduction of the channel bandwidth with simultaneous increase of transmissive power will not lower the transmissive capacity of the channel. Methods of coding the physiological information, including such systems as cardiophonic pulse transmission; single-channel three-parameter coding and five-parameter computer-assisted coding, are discussed.

The paper is concluded with a brief review of the types of radiotelemetering systems in the laboratory conditions, aboard the spaceship and the complete ground-to-space system, which is capable of receiving and transmitting information employing translators. It is stressed that further development of biosensors and the medical instrumentation associated with them will largely depend on miniaturization and general progress in electronics as well as in medicine.

Author


[Translator's note: This is an original abstract and not a copy of the English "summary" printed with the article.]

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# PROBLEMS OF SPACE MICROBIOLOGY AND CYTOLOGY

N. N. Zhukov-Verezhnikov, I. N. Mayskiy, V. I. Yazdovskiy, A. P. Pekhov, N. I. Rybakov, N. N. Klemparskaya, A. A. Gyurdzhian, G. P. Tribulev, N. P. Nefed'yeva, M. M. Kapichnikov, I. I. Podoplelov, V. V. Antipov, I. S. Novikova, and V. Ya. Kop'yev.

The historical achievements of Soviet scientists in the study and conquest of cosmic space have opened up prospects for solving a number of new scientific problems, not only in mathematics, physics, chemistry and astronomy, but also in biology and in particular microbiology and cytology. We can now speak with confidence of the birth and development of space microbiology and cytology as new scientific disciplines forming part of space biology.

Space microbiology, which originated as an experimental discipline in the Soviet Union, is a natural continuation and development of trends traditional of Russian microbiology as represented by the work of I. I. Mechnikov, D. I. Ivanovskiy, V. L. Omelyanskiy, N. F. Gamaley, S. N. Vinogradskiy, and in the Soviet period by the investigations of V. N. Shaposhnikov, A. A. Imshenetskiy, P. F. Zdrodovskiy, N. A. Krasil'nikov, Ye. N. Mishustin, M. A. Morozov, V. D. Timakov, P. G. Sergiyev, V. M. Zhdanov, G. V. Vygodchikov, M. V. Fedorov, V. L. Troitskiy, Z. V. Yermol'yeva, S. N. Muromtsev, M. P. Chumakov, and others. The beginning of space cytology can also be described in similar terms.

In the fields of space microbiology and cytology the following problems are being studied:

1) The biological activity of terrestrial microorganisms and somatic cells (in monolayer cultures) when exposed in outer space.

2) The genetic effects produced by factors of outer space.

3) The simplest forms of extra-terrestrial life.

4) The problem of drift of terrestrial microorganisms to the surface of other planets and also of transfer of microbes from the planets to Earth.

These problems arose in connection with the possibilities opened up as a result of the penetration of the first Soviet satellites into outer space.

During the last few years many of these problems have been the subject of intensive experimental research carried out in space craft; these yielded the first practical data and form the basis for further research (Zhukov-Verezhnikov et al., 1959, 1960, 1961; Vishniac, 1960; Anders, 1961; Sagan, 1961).

## Study of the Life Conditions of Microorganisms and Somatic Cells when Exposed in Outer Space

This problem sprang from the practical need to ensure the safety of manned flight in cosmic space.

The experiments carried out by Soviet investigators on dogs in rockets and space ships have shown that residence of highly organized animals in outer space does not appreciably affect the performance of their basic physiological functions (V. I. Chernov, V. I. Yakovlev, 1958; B. G. Bugrov et al. 1958; A. M. Galkin et al. 1958). The results of these experiments also indicated that if the animals are provided with optimal conditions of life they are able, during flight, to withstand quite successfully exposure to a number of factors (noise, vibration, weightlessness, etc.). Thus, these observations revealed in principle the possibility for animals to remain in space and endure orbital flights around the Earth without any substantial disturbance in their physiological functions.

However, it was necessary to decide whether the tolerance to space flights shown by higher animals was due to the work of their compensatory systems which eliminated the effects of primary damage to cell structures, or whether this primary damage was absent altogether. It was only possible to solve this problem by using cultures of cells or of unicellular organisms, for example, microbes. Moreover, the use of cells might prove useful in studying the biological effect of cosmic radiation.

Study of the biological effectiveness of the corpuscular components of cosmic radiation in the form of protons, $\alpha$-particles (helium nuclei), and nuclei of heavier elements characterized by enormous energies and considerable penetrating ability, is one of the most important problems in current research. The complexity of the problem is due to the great difficulties involved in simulating the factors of space flights under terrestrial conditions.

The investigations carried out in the U. S. S. R. and the U. S. A. on artificial earth satellites and rockets have shown that around the Earth there are two belts with a high level of radiation (Vernov and Chudakov, 1960; Vernov, Chudakov, Lebedinskiy and Ivanenko, 1960; Van Allen, 1960). The outer radiation belt, located in the equatorial plane

at altitudes from 14,000 to 50,000-55,000 km from the Earth, has a broad electromagnetic spectrum. The stream of electrons in this belt produces a surface dose of about $10^6$ roentgen/hour. The inner belt, at 600 to 5,000 km from the Earth, is characterized by harder radiation, the dose of which under the protection of 1 g of light material per $cm^2$ is about 10 r/hour.

It has been established that the boundaries of the radiation belts are not stable. Therefore a great deal of research has still to be carried out before we can forecast the precise level of cosmic radiation for a flight of given duration.

When penetrating into the atmosphere, high energy primary cosmic ray particles collide with nuclei of atoms of the atmosphere, producing showers of secondary cosmic radiation. Consequently the radiation close to the Earth consists of a mixture of primary and secondary particles.

Finally, the composition and energy of the particles of primary cosmic radiation differ considerably from those of the radiation under terrestrial conditions. Even with the most modern accelerators it is not possible to produce particles with energies comparable to the enormous energies of cosmic radiation particles.

All this shows that initial, fundamental and conclusive information on the biological effectiveness of cosmic radiation can be obtained only from direct experiments in which biological material is exposed to conditions prevailing in outer space, since it is not possible to reproduce the whole spectrum of primary cosmic rays under terrestrial conditions. Such experiments on highly organized animals are at present restricted by the difficulties involved in supplying food and oxygen and in general, by the difficulty of creating conditions which would permit prolonged flight experiments in outer space. The best results have been obtained by the use of microorganisms, human cells in single-layer tissue culture, and flaps of human and rabbit skin. Bacteria have also been used in attempts to ascertain not only the degree of viability but also the possibility of reproduction of organisms in cosmic conditions.

The results of the experiments carried out in the second Soviet satellite launched on August 19, 1960, were reported in a paper by N. N. Zhukov-Verezhnikov, I. N. Mayskiy, V. I. Yazdovskiy, A. P. Pekhov et al. (1961). We shall compare these findings with the results obtained from the first manned space flights and endeavor to analyze certain new findings. As is known, microbiological and cytological experiments on satellites pursued the following aims:

a) determination of the number of viable cells in microbe suspensions;

b) determination of the number of viable phage particles in phage filtrates;

c) investigation of the viability of human somatic cells in tissue culture;

d) study of the viability and take of human and rabbit skin flaps on autotransplantation;

e) investigation of bacterial reproduction with use of automatic instruments and transmission of the results by the system of telemetry;

f) study of the genetic influence of the factors of space flight on bacteria.

The following typical organisms were used in experiments designed to study the survival of cells in bacterial suspensions exposed in space: 18-hour agar cultures of Escherichia coli K-12 ($\lambda$), Aerobacter aerogenes 1321, E. coli B, Staphylococcus aureus 0.15 and suspensions of Clostridium butyricum spores.

After return of the test samples from flight, the number of viable cells in them and in the control samples was determined by inoculation by the agar layer method. Retention of viability in Cl-butyricum was determined by establishing the gas forming ability of the bacteria in dilutions. The results showed that there are no marked differences in the concentration of surviving bacteria in the experimental (which made the flight) and the control samples.

The use of bacteria in carrying out the biological program in the second space ship was not confined to the gathering of information on the viability of the bacteria, which could be established only after return of the samples to Earth. In the second and later Soviet satellites Cl. butyricum was exposed. These organisms were used to check the methods of automatic recording of the life function of microorganisms in special apparatus - the bioelements. It was necessary to develop such a method for the purpose of subsequent study of the maximum duration of cell viability without returning the samples to Earth; this is exceptionally important in experiments with non-returning artificial earth satellites designed for flying over long periods in various zones of the solar system.

A further advantage of automatic instruments for the biological charting of cosmic space is that they are small in size and weight and the bacterial spores with which these instruments are "charged" may be

stored in them for years without the necessity for replenishing the medium with fresh nutrient substances. The bioelements are able to record and transmit to Earth information on the viability and certain physiological functions of spore bacteria during prolonged flights, being brought into action by signals from Earth or by a programming device on board the satellite.

The ability of bacteria to generate gas, which is easy to record, formed the basis of the design of special instruments (bioelements) intended for the study of the life functions of bacteria under space conditions. These instruments are designed to record the generation of gas resulting from the life functions of the organisms and to signal this information back to Earth. Below, the potentialities and properties of the bioelements will be discussed in greater detail.

The developed bioelement AMN-1 is a metal cylinder consisting of two chambers, one being the inoculation[1] with a volume of 1 $cm^3$, and the other the culture chamber (volume 10 $cm^3$). A culture of the organisms is introduced into the inoculation chamber and the culture chamber is filled with nutrient medium. The chambers are separated by a transverse glass partition. The inoculation chamber on the top, and the culture chamber underneath, are bounded by flexible diaphragms. A striking pin fixed to the diaphragm and touching the glass partition is arranged vertically in the inoculation chamber. For automatic inoculation a striking device is screwed on to the inoculation chamber and when the striker of this device hits the diaphragm the pin smashes the glass separating the chambers and thus inoculates the spores. The gases formed in the apparatus as a result of the process of living of Clostridium butyricum bend the diaphragm of the culture chamber; this closes an electric circuit and a signal on the functioning of the instrument is transmitted to Earth by telemetry.

In experiments carried out on Soviet spacecraft the electrical circuit is automatically closed in accordance with a preset program, the relay of the striking devices of the bioelements is activated and the microorganisms are inoculated into the nutrient medium. Response of the relays caused immediate blocking of these, opening the circuit (so as to reduce the power consumption). At the same time, the electrical circuit used ensures:

1) reception on Earth of information indicating that the striking device has worked and

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[1] Accordingly, in the initial design of the bioelement, ejection with a piston was envisaged.

2) switching on of a thermostat; from this moment onwards the temperature of the thermostat begins to increase. Thus, the information received about the functioning of each bioelement consists of the following signals:

a) operation of the striking device (inoculation of the microorganisms into the nutrient medium);

b) characteristic of the temperature in the thermostat;

c) flexing of the membrane of the culture chamber (formation of a sufficient amount of gas as a result of the metabolism of the organisms).

The use of bioelements automatically recording the biological activity of anaerobic spore-forming bacteria made it possible to obtain initial information on the possibility of reproduction of bacterial cells when exposed in space.

After dismantling the bioelements that had returned, no visible changes were observed in the cultures of Clostridium butyricum exposed in space, as compared with the controls. In every respect (morphology, biochemistry, fermentative powers, etc.) the bacteria isolated from the test and control bioelements were identical. Thus a check on the fermentative ability of the cultures which had returned from outer space showed that the amount of gas with 100 ml nutrient medium after 48 hours incubation varied from 0.582 to 0.700 g as against 0.657 to 0.740 g in the controls. New types of bioelement designed to the specifications of our team are capable of recording automatically not only gas formation but also other forms of vital activity of microbes. This makes it possible to use the bioelements in attempts to determine the presence of life on the planets. For this purpose the bioelements will be fitted with trapping apparatus. The findings of Soviet investigators on the effect of cosmic factors on microorganisms were later confirmed by American workers (in relation to tolerance), who found that spore-forming bacteria (Clostridium sporogenes) remained viable in experiments conducted in the earth satellite "Discoverer 17", the flight of which coincided with an intense solar flare (Bulban, 1961).

In the first series of experiments carried out on the second space ship, bacteria phage was used in addition to bacteria. Although it is known that phage are fairly resistant to ionizing radiation, they were used in the experiments in the belief that the effect of the corpuscular component of cosmic radiation (charged heavy particles, neutrons) must be greater than the action of X- and $\gamma$-rays. The phage selected for the experiments was phage 1321 and T-2, on the grounds that these phage have been most studied. These phage lyse E. coli B and A. aerogenes 1321 respectively.

The experiments showed that the number of viable phage particles in the test and control samples was identical, thus indicating that the stay of phage in outer space does not affect its viability since the number of viable phage particles per milliliter of phage filtrate was the same as in the controls. It is important to note that similar results were obtained in experiments with the 4th and 5th space ships.

As has already been stated the use in space experiments of single-layer cultures of human cells, despite great technical difficulties has, as already stated, substantial advantages, since it permits detection and study of the effect of factors of outer space which cannot be determined in the whole body because of compensatory adjustments ensuring rapid replacement of the injured or dead cells. As was shown in laboratory experiments, a mono-layer culture of human cells is highly suitable for studying the effects of many factors of genetic changes, in particular ionizing radiation. It is known that the radiation sensitivity of cultivated human cells is hundreds of times greater than that of micro-organisms (Puck, Morkovin, Marcus, Cilciura, 1957, and others). Therefore the use of this model in experiments on Soviet space ships was prompted by the assumption that even the slight effects of the space factors can be detected in cell cultures.

The experiments carried out on the Soviet satellites were essentially aimed at obtaining information on the following aspects:

a) in vitro survival of mono-layer culture cells;

b) viability of cell cultures;

c) ability of human cells to grow and reproduce when the culture is subsequently transferred to normal conditions;

d) the in vitro character of growth of cell colonies, cell morphology and antigenic properties.

The initial cell culture in the experiments with the second Soviet satellite was a 7-day cloned Hela cell culture with subsequent use of normal human fibroblasts.

The experiments justified the conclusion that stay of mono-layer cultures of human cells in outer space does not produce any profound changes as compared with the controls, that the bulk of the cells of the test cultures remain on the glass, and that the cultures remain viable. On transplantion the cells of the test cultures grew and reproduced to the same extent as did the cells of the control cultures.

Subsequently all the initial test and control cultures which had shown primary growth were cultivated for a long time (more than one year)

in standard conditions in the form of lines of experimental and control cultures. In each passage the following were studied: the character and potential of growth, the ratio of living to dead cells, and in a number of passages their antigenic and morphological properties. It was found that in all the passages the biological properties of the cultures did not differ in any important respect. The test and control cultures retained their capacity for active growth and reproduction from the very first passages.

In later experiments in other satellites, including "Vostok-1" in the cabin of Yu. A. Gagarin the range of strains of human cell cultures was extended. Two strains of Hela cancer cells were used, namely a strain which had already travelled on the second satellite, and an original strain kept before the flight in the usual laboratory conditions. These experiments also made use of strains of normal human cells (fibroblasts and amnion cells). Again, similar investigations of the basic biological properties of the test and control cultures did not reveal any appreciable differences between them. Study of these cultures is continuing.

These findings thus show that the travel of human cells in the form of mono-layer cultures in space ships does not affect their viability or basic biological properties.

The results of study of the effect of outer space on the viability and subsequent take of human and rabbit skin flaps on autotransplantation are also of fundamental importance. It was considered important to establish the effect of space factors on the viability of isolated tissue systems for specimens with different levels of biological organization.

Skin flaps from three volunteers were exposed in the second Soviet space ship. Study of the take of their skin and the clinical observations made after autotransplantation and also the results of cultivation of the skin fragments in tissue cultures showed that the skin flaps remained viable after having been in outer space.

Thus the study of the biological effects of outer space with use of microbiological and cytological specimens yielded results of great practical importance in the period of preparation for the first flight by man into space. In other words, the techniques employed to make a careful investigation of the orbits followed by Yu. A. Gagarin and G. S. Titov included the use of microbiological and cytological specimens, as described above. It was found that tolerance of space flight shown by higher animals is due not only to adaptive-compensating ability of the body as a whole, but to primary invulnerability of its cells. Naturally, this conclusion applies to the orbits studied and the periods during which the space ships were in these orbits and were exposed to the given radiation conditions.

These initial experimental data will gain in significance with continued and more detailed research aimed at the further mastery of outer space, in particular during preparations for long space flights by man. Although some experiments will be carried out under laboratory conditions, nevertheless the main results will be obtained by experiments carried out during actual space flights. To make a conclusive assessment of the biological effect of cosmic radiation it will be necessary to obtain information on the distribution of ionization in living matter and the number of tracks formed by the particles, the density of ionization along the tracks, the number of tracks and the structural damage to the cells, etc. The increase in knowledge of the effect of cosmic radiation will be helpful in devising reliable methods of radiation protection during space flights.

In addition to radiation, weightlessness is also fundamental to the problem under discussion. Weightlessness is a phenomenon which has received no study at all in the biological sense. Yet present-day terrestrial organisms, during evolution, developed under the influence of the most universal force - gravity. The influence of gravity explains the most important morphological and functional peculiarities of organisms, in particular such features as the arrangement of the limbs, the distribution of masses around the center of gravity, their interrelation, etc. It is possible that the reciprocal arrangements of a number of cell structures came about under the influence of this factor. It is therefore very important to obtain information on the effects of gravity and weightlessness on the internal life of the cell, in particular on the processes of diffusion and osmosis, the reciprocal arrangements and integrity of intra-cellular components, the processes of synthesis of vitally important substances, etc.

The difficulties of carrying out experimental work with the corpuscular components of cosmic radiation under terrestrial conditions have already been mentioned. In precisely the same way, it is still not possible under terrestrial conditions to make a prolonged study of weightlessness although its role and influence on the body in sustained flights are unquestionable.

It would seem that this problem will in the future have to be tackled by studying the biological activity of microorganisms and somatic cells on prolonged exposure in space. There is no doubt that it will be very important for this purpose to use automatic instruments of the bioelement type, not only for studying the vital activity of bacteria but also for investigating somatic cells and tissues.

Investigation of the Genetic After-Effects Produced by Outer Space Conditions

Study of the genetic effects of residence in outer space is of great theoretical and practical importance.

Besides bearing a direct relation to the genetic safety of prolonged manned flights in space, this problem has also relevance in another respect. Thus, for example, it is very important to ascertain the possibility of utilizing the corpuscular components of cosmic radiation for "radiation selection" of useful forms of microorganisms. However, study of this problem involves great difficulties since the data obtained by examination of the genetic effect of ionizing radiation on organisms under terrestrial conditions cannot a priori be applied to cosmic parameters. Therefore the necessary information must be obtained from experiments involving the space flight of the test samples.

In selecting the microbiological specimens for the biological program to be carried out in Soviet space ships, the specimens chosen were those suitable for recording hereditary changes induced not only by high-activity radiation, but also by exposure to low dose radiation. The idea in exposing microbiological objects differing in radiosensitivity was to detect uncommon factors, i. e., those which could not be foreseen since they are absent under terrestrial conditions. As can be seen from the material presented, enteric bacteria including lysogenic bacteria and phage, were employed on the second and also the other Soviet satellites in a series of genetic experiments.

The bacterial cells of the majority of species are highly resistant to the effect of ionizing radiation. Mutagenic doses of ionizing radiation are also highly effective. Auxotrophic mutations of enteric bacteria, i. e., mutations resulting in loss of the ability of the bacterial cell to carry out independent synthesis of amino acids or vitamins, can according to a number of workers be reliably induced by doses of the order of tens of thousands of roentgens (Braun, 1953). Doses of thousands of roentgens are necessary even for a considerable reduction in the frequency of recombinations in the crosses of auxotrophic bacterial variants (Wilson, 1960).

Lysogenic E. coli K-12 ($\lambda$) was chosen because of the remarkable ability of these organisms to produce phage after exposure to a number of factors including ionizing radiation. An important technical point of the experiments is that while for instance the induction effect of ultraviolet radiation can be eliminated by visible light, the induction effect of ionizing radiation is irreversible. The duration of the period after which lysogenic bacteria produce phage in response to the effect of ionizing radiation (latent period) does not depend on the radiation dose but the number of induced bacteria is directly dependent on dose. This

makes it possible to determine the number of free phage and induced bacteria in relation to specific doses. Published findings (Marcovich, 1956, 1958) and the results obtained in our laboratory, show that lysogenic bacteria produce phage in response to such low doses of ionizing radiation as 1 r and even lower. Lysogenic bacteria spontaneously produce phage; however, there are now available methods (Marcovich, 1956) making it possible to count even small numbers of the induced phage particles and to differentiate them from spontaneously produced phage particles by means of anti-phage serum. These methods were applied to analyze samples of lysogenic bacteria exposed in outer space.

In discussing the nature of phage, a number of workers, including the authors of this paper, have repeatedly suggested (Zhukov-Verezhnikov, 1936; Zhukov-Verezhnikov and Pekhov, 1961) that the formation of phage is connected with genetic processes in bacteria. Recently this problem was again studied by Northrop (1958), who compared the production of phage by lysogenic bacteria with the formation of antibiotic-resistant mutants and again supported the idea that these processes are similar. In fact, if we start from the premise that mutations are changes in hereditary properties mostly of a pathological nature, then the lysogenicity of bacteria can be interpreted as a pathological hereditary reaction of the transformation type. The current state of knowledge of phage suggests that the ability of lysogenic bacteria to produce phage under the influence of radiation is hereditary.

Thus, experiments with lysogenic bacteria served as a convenient model for studying the genetic effects of cosmic radiation, particularly since on the eve of the first flights by man into space the main problem was that of recording genetic effects induced by low doses.

In experiments with the second Soviet satellite the results of study of the effects of outer space on phage production were determined by counting the number of phage particles produced after inoculating on to fresh nutrient media the lysogenic bacteria exposed in outer space, and by comparing the findings with the results of inoculation of control bacterial samples kept during the experiment in the laboratory. The test object was the lysogenic strain E. coli K-12 ($\lambda$).

The results indicate that the number of phage particles produced by lysogenic bacteria after exposure in outer space was somewhat greater than the number of phage particles detected in the cultures of the control bacterial samples (spontaneous phage production). It was very difficult on the basis of these findings to say whether there was any induced phage production, since precise determination was hampered by the constant presence in the cultures of lysogenic bacteria of phage particles formed as a result of spontaneous lysis of the individual cells. Therefore, in later experiments on space ships the technique of detecting induced phage production by E. coli K-12 ($\lambda$) was modified to increase its

effectiveness. Since cultures of lysogenic bacteria always contain a certain number of free phage particles, the technique employed was based on inactivation with specific anti-phage serum after the end of the latent period in the development of the induced phage particles (Marcovich, 1956). The following procedure was adopted. Suspensions of cells of E. coli K-12 ($\lambda$) exposed in outer space were inoculated into meat-infusion broth and grown for a period corresponding to the length of the latent period of the lambda phage. Then antiphage serum was added in a concentration sufficient to achieve complete inactivation of the free (spontaneous) phage particles. After 10 minutes the action of the serum was arrested by diluting the bacteria suspensions, streptomycin was added and the samples were plated by the agar-layer method with streptomycin-resistant bacteria of an indicator strain of E. coli.

The results of the experiments with the fifth Soviet satellite show that lysogenic bacteria did not produce phage after exposure in outer space. The effectiveness of the technique employed was checked in parallel experiments on determining the induction of phage with low doses of ionizing radiation in laboratory conditions. It was found that by this method even a slight increase in the production of phage by lysogenic bacteria induced with low doses of ionizing radiation can be recorded. Thus, it was possible to assume that exposure of lysogenic bacteria in outer space under the experimental conditions existing in the satellite did not affect their ability to produce phage particles. Somewhat different results were obtained from flights along approximately the same orbit but of longer duration. Thus the samples of lysogenic E. coli K-12 ($\lambda$) placed in the cabin of G. S. Titov ("Vostok-2") showed that the number of cells forming phage was slightly in excess of the samples left in the laboratory.

It is emphasized that what is being discussed is comparison of data from the test samples with those of the controls which remained in the laboratory. However, for technical reasons this control sample cannot be considered fully acceptable since in this respect the batch of control samples carried to and from the launching pad is important. Study of these samples showed that the number of bacteria producing phage is not lower than in the exposed samples. This indicates that the 25-hour orbital flight of satellites of type "Vostok-1" and "Vostok-2" does not have a detectable effect on the elementary poorly compensated genetic process as reflected in the production of phage by lysogenic bacteria. Although the genetic effects of vibration, acceleration and weightlessness must be taken into consideration, this is not as important as when analyzing the genetic effects of flight for multicellular animals and plants. The authors assume that lysogenic bacteria, on being immersed into a liquid medium, are more protected against the sudden accelerations produced by passage into and out of the weightless state. In this sense such specimens provide a better idea of the real biological effects of cosmic radiation than do multicellular ones, and can be used to

differentiate the factors involved in the overall genetic effects in space flight. Such differentiation is highly important. The point is that vibrations and accelerations on take-off and landing are largely unrelated to the duration of the flight. However, duration is now the main problem and here the prime factor is cosmic radiation which is capable of causing cumulative genetic changes. It is also necessary to determine whether a prolonged state of weightlessness can in itself produce genetic effects. This can be done by a microcentrifuge installed on satellites which would simulate the force of gravity for the control sample cells.

Returning to the analysis of the importance of selection of genetic specimens, we would note that after exposure of phage filtrates in space ships the possibilities of detecting mutations in the form of plaques were slight, since the latter are highly resistant to radiation. Nonetheless, the possibility of a direct hit by cosmic particles of the phage corpuscles was not ruled out. In addition, in outer space there may be unknown factors capable of inducing this form of phage mutation. The experiments with the second Soviet satellite were concerned with the test samples of phages 1321 and T-2. Several thousands of plaques from each sample were examined, but no changes in plaque morphology were detected either in the test or control samples. The spots examined had the structure typical of each phage species. Now a few words on the results of investigations of other genetic changes in bacteria. Previously published work of the authors showed that no increase in the frequency of mutations of E. coli K-12 and E. coli "B" is observed after space flight. However, on examination of one of the samples of E. coli K-12 present in G. S. Titov's cabin ("Vostok-2"), N. N. Klemparskaya found dissociative phenomena with the sample taken into space showing a somewhat greater tendency to such phenomena. These cultures are still under study.

Thus, the results of the experiments carried out on Soviet space ships show that there are no fundamental changes in the hereditary properties of bacteria and phage when kept in the conditions of outer space. The experience gained during these experiments indicates that the most promising line of study of the genetic dangers of outer space with use of microbiological objects is to carry out experiments with lysogenic bacteria. Being a suitable model lysogenic bacteria will play a leading role in future investigations. Improvements in this method will depend on the progress made in designing the necessary automatic apparatus for determining the phage particles induced directly during flight.

## Investigation of the Simplest Extra-Terrestrial Forms of Life

It is generally known that, according to the theory of S. Arrhenius, transfer of living matter from one planet to another is possible.

Backed by the latest technical advances, a research program must be carried out in order to ascertain whether living organisms are present or absent in interplanetary space and on the nearest planets. This program must include as a minimum requirement search for spores and investigations in the field of organic chemistry in order to detect in outer space complex organic compounds or the chemical precursors of these compounds.

As is known, terrestrial organisms are protected from cosmic radiation by the atmosphere and the magnetic field of the Earth. This protection is absent, for example, on the Moon and therefore if we postulate the presence of microorganisms on the various planets, they must possess reliable protective mechanisms. It is known that in terrestrial conditions there are organisms which can live or are in a state of anabiosis at very low temperatures, high pressure, etc., that is, in very severe conditions. These facts suggest that it may be possible to detect life at high or low temperatures, minimal humidity and other unfavorable conditions. The possibility of adaption of microorganisms to ionizing radiation outside the Earth's atmosphere is indicated by the great differences in the radiation-sensitivity of different species of terrestrial organisms. There is some reason to believe that a reliable form of protection of spores is provided by meteors. In other words, to answer the questions raised we need material obtained from interplanetary space, from the surface of the Moon or other planets. Because of the tremendous speed at which meteors move in space, it is extremely difficult at the present state of development to obtain interplanetary substance.

Many specialists (Lederberg, 1958 and others) consider that, owing to the absence of atmosphere on the Moon, meteors have been accumulating there for a very long time.

Investigation of lunar dust will probably make possible the study of the history of the universe, in the same way as the history of the Earth is studied by analyzing geological strata.

It is obvious that together with search for the spores of microorganisms in cosmic dust, it will be necessary to investigate it for the presence of organic substances - signs of living matter - particularly since some astrophysical findings indicate that the atomic composition of cosmic matter is suitable for the synthesis of such substances. Recently M. Briggs (1960), employing infrared spectroscopy, obtained information on the spectrum of Mars which he is inclined to interpret as evidence of the presence in the various zones of this planet of complex organic substances.

A. I. Oparin (1958) proposed the most probable schemes of historically primary synthesis of organic substances. On the basis of these schemes there is every reason to believe that investigation of cosmic

(lunar) dust will lead to the detection of lines of synthesis characteristic of the principles of conversion of inanimate to living matter in general.

It is urgently necessary to make a detailed and deep study of the microbial population in the upper layers of the atmosphere. We need to solve the highly complex problems involved in designing automatic apparatus, conveyance of samples to Earth and identification of bacterial cultures when isolated. However, the first need is to attempt to use bioelements (suitably modified in design) to collect and investigate on the spot the material on the planets and signalling the results of this investigation back to Earth. Thus it may be possible, for example, to find out whether the Moon is sterile as a majority of specialists consider, or whether life exists on it in the form of microorganisms. Bioelements with a developed system of automatic culturing of material and a system of radio signals on reproduction and vital activity of microorganisms, are highly suitable for such attempts and the information now available on the physiology of various microorganisms makes possible the selection of suitable nutrient media.

## Problem of Transfer of Terrestrial Microorganisms to Other Planets and also Transfer of Microorganisms from the Planets to Earth

For the time being this problem is still only of practical importance due to the fact that terrestrial microorganisms can be transported from Earth to other planets by spacecraft. It is easy to see that failure to recognize this possibility would greatly complicate future efforts to detect microorganisms on other planets. Therefore, with the development of space flight it became necessary to sterilize the spacecraft.

As is known, the second Soviet space rocket launched on September 12, 1959 and landing a pennant with the State coat of arms of the U. S. S. R. on the Moon, was initially freed of organisms. The work begun in this direction must be continued when other programs materialize.

Another aspect of the problem is that spacecraft may be contaminated beyond the confines of Earth although the actual degree of such contamination cannot be accurately determined since the properties of nonterrestrial forms of life can be described only in the most general terms. Nor should it be forgotten that all major geographical discoveries on Earth have resulted in spreading of dangerous microbes, for example, the agents of plague, cholera and yellow fever. Mankind has repeatedly paid a heavy price in hundreds of millions of lives because of ignorance of the microbiological background in newly acquired (unknown) natural regions. We must prevent penetration into the terrestrial system, permanently enclosed by the atmosphere and magnetic fields, of substrates artificially brought in from outside and about which nothing is known in

terms of their microbiological safety. Abstract conclusions are dangerous here, and only direct experiments can decide whether or not microorganisms are present in outer space and indicate their nature. This makes it necessary to adopt special measures in collecting and treating material from outer space. However, the main task in this respect is preliminary investigation of the planets with automatic instruments to see whether microorganisms are present there. As shown above, all the conditions for accomplishing this task exist.

Space microbiology and cytology only recently became genuine branches of science. However, the unprecedented pace of scientific progress in the U. S. S. R. has created such technical conditions for biological experiments that in a few years it was possible to gather factual material determining the main features of these new specialized disciplines. It is particularly important to develop intensively the main lines of space microbiology, bearing in mind that when man encounters new worlds, microbiological investigations may perhaps be of crucial importance.

Summary

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Microbiological and cytological investigations carried out in the Soviet satellites Vostok-1 and Vostok-2 were intended for solving the following two problems of biology: 1) study of conditions of life in outer space (viability of microorganisms and somatic cells and reproduction after exposure); 2) study of the genetic effects caused by space flight. Biological material of different levels of organization was studied: bacteria, phages, cells in tissue culture (Hela cancer cells, fibroblasts, amnion cells) and skin flaps which were regrafted to their donors after flight.

In addition, special automatic instruments, bioelements AMN-1, were used to record the biological activity of Bac. butyricum during the flight.

No essential changes were observed in comparison with controls in microbiological and cytological specimens used in the space flights. It assumed, therefore, that space flight conditions have no unfavorable effect on highly organized, multicellular organisms of more highly developed animals or living cells.

Only in one experiment in Vostok-2 was a slight increase observed in the induced phage production of lysogenic bacteria. The dissociative phenomena in the culture E. coli KK-12 increased somewhat. It is important to study further the biological characteristics of space and to improve experimental techniques, particularly the wide application of such biological high-sensitivity indices as induced phage production of lysogenic bacteria.

The possibility of existence of life in outer space and methods of detecting it are considered from the point of view of fundamental biology, emphasizing the necessity of taking measures to prevent contamination of celestial bodies with terrestrial microorganisms or vice versa.

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# THE POSSIBILITY OF EXISTENCE AND METHODS OF DETECTING EXTRATERRESTRIAL LIFE

A. A. Imshenetskiy

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The extremely rapid developments in physics, engineering and chemistry in the Soviet Union have enabled a start to be made on the conquest of space by man. Biologists are faced with new tasks and many of these pose a number of questions. Can terrestrial microorganisms be carried into cosmic space? Can single cells stand the effect of the physical factors which will act on them in cosmic space? What type of life is possible on other planets? What principles can be applied to methods of detecting these forms of life? These and other problems cannot fail to attract the interest of biologists all over the world. For many years all the hypotheses relating to these problems have been purely hypothetical. For the first time in the history of mankind it is now possible to solve them experimentally.

It is difficult to overestimate the importance of the results which can be obtained in these investigations. Possibly they will permit us to become acquainted with new forms of life, throw light on individual stages of formation and development of life; enable general and specific comparisons of terrestrial forms of life with organisms inhabiting other planets to be made. However, even if no living beings are found it is more than likely that organic substances will be detected, the composition of which will help in the understanding of the evolution of abiogenic organic chemistry.

It is quite obvious that at present it is not possible, on the basis of experimental data, to discuss the nature of life on other planets and methods of detecting it. However, this does not mean that we should not outline methods of investigation in this field. This communication is a modest attempt by a microbiologist to elucidate some of the questions relating to this problem.

The study of the biosphere, which was initiated by the outstanding biogeochemist V. I. Vernadskiy, confronted biologists with the necessity of establishing the upper and lower limits of the biosphere. As regards the lower boundary, the discovery of microorganisms in petroleum waters emanating from the depths of the earth, as well as the presence of

various terrestrial beings in soil taken from the bottom of oceans, lead to the assumption that the limit at which living creatures can be detected is 10 to 11 km below sea level. It is much more difficult to establish the upper boundary of the biosphere. Even the samples taken a long time ago in the United States at an altitude of 20 km by means of an instrument launched from the balloon "Explorer II" revealed the presence of bacteria and fungi at this altitude. So far, the upper boundary of the biosphere has not been established since there are no irrefutable results of investigations which would prove or disprove the existence of cells of terrestrial microbes at an altitude of 200 km.

When the presence of microbes at high altitudes is discussed, generally one returns to the views of S. Arrhenius, which are not always correctly interpreted. In this theory, two entirely unconnected problems have to be distinguished. The first of these is the problem of the origin of life and the second is the possibility of transportation of spores through cosmic space and from one planet to another. Arrhenius considered that life is eternal in the same way as matter is eternal and that the spontaneous generation of living beings is impossible. This is what he wrote on the subject: "Up to now, it was believed that life formed from inorganic matter as a result of the so-called process of spontaneous generation. However, in the same way as the goal to self-formation of energy, "perpetuum mobile", had to be abandoned in view of the negative experimental results, it is also likely that the impossibility of observing spontaneous generation of life will lead to the view that such a process is absolutely impossible. In order to answer the question of the possible generation of life on the planets, it is necessary to revert to studies on pansperm; I studied this subject in accordance with the present state of science, combining it with the study of the contribution of radiation to this process".[1]

Thus, negating self-formation, Arrhenius explained the appearance of life on planets by supposing that it was transferred from one planet to another. The problem of the beginning of life remains open since life is eternal. The idea of the eternal nature of life was widely held by many, including Libich, Helmholtz, Wagner, Richter and Thomson.

Present-day information in the field of natural sciences provides a working hypothesis for the origin and evolution of life starting from the formation of living matter from non-living matter. We will not dwell on this problem since it has been very well described in the papers of a symposium published in the Soviet Union, devoted to the origin of life, as well as in the well known book by A. I. Oparin.

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[1] S. Arrhenius. Formation of Worlds, Odessa, 1908, IV.

Views have been expressed that the assumptions made by Arrhenius on the travel of microorganisms over large distances in cosmic space are unlikely and even impossible. I believe that there is no justification for confusing these two problems. The assumption that living germs can be transported in cosmic space is not impossible or erroneous from the theoretical point of view. Even now, the development of modern science permits a changing over from general considerations to experimental verification of the possibility of carrying microorganisms from one planet to another. On the basis of purely theoretical considerations, Maxwell (in 1873) expressed the assumption that daylight exerts a pressure. P. N. Lebedev, in his paper "On the force of repulsion of light-emitting bodies", published in 1891, proved that a pressure is exerted by light. In further work Arrhenius considered the importance of this pressure from the point of view of cosmic physics. It is probable that, due to the pressure of light, the smallest dehydrated but viable microorganisms can travel in space over great distances. Thus, there is no justification for ruling out the possibility of the "exportation" of particles from the Earth as well as "importation" of spores to the surface of our planet. Even if published reports on the presence of microbes in meteorites are not confirmed, it does not mean that we have the right to negate the possibility of existence of living organisms in space.

The variety of ecological conditions which exist on various planets and in space determine to a certain extent the nature of possible forms of life. The only approach to this problem is through the study of cosmic ecology. However, in assessing this problem we should start off from "terrestrial" concepts on the features of metabolism of single-cell bodies as well as from the existing definitions of life. Frequently, the view is expressed that if life exists on other planets, its forms should differ relatively little from the forms of life on Earth. Taking into consideration the extreme flexibility of single cells, it is easier to assume that they can adapt themselves to conditions of life which are very unusual, from our point of view, than to look for basically new forms of life which, for instance, do not contain carbon or water.

Considering the possible effects of various external factors, it is necessary in the first instance to discuss the effects of temperature. If a very large number of microorganisms withstand extremely well exposure to the temperature of liquid helium, i.e. a temperature approaching absolute zero, then there is no reason to assume that single cells will not survive exposure to low temperatures. However, the problem of the possibility of multiplication at very low temperatures remains open since the minimum temperature at which terrestrial bacteria multiply is in the neighborhood of $-10^{\circ}$ C.

With regard to elevated temperatures, thermophilic forms of life developed during the process of evolution as a result of their adaptation

to temperatures of 80-85$^{\circ}$ C. It was experimentally established that some thermophilic spore-forming bacteria could withstand boiling for 4 to 5 days. If highly stable thermophilic forms of life developed on Earth where there are relatively few ecological regions which are favorable from this point of view, there is no theoretical basis to rule out the possibility of the adaptation of microorganisms on other planets to

higher temperatures, reaching 140-150$^{\circ}$ C. These temperatures may exist at the surface of some planets, particularly in water with a high salt concentration. Life at elevated temperatures is quite possible and highly perfected mechanisms which ensure stability of proteins at high temperatures may exist.

It is well known that under experimental conditions in laboratories bacterial spores are not destroyed when they are under vacuum or when they are dehydrated. With regard to the effect of radiation on the microbial cell, it is necessary to point out that the intensity of ionizing radiation in space cannot be considered a barrier to the development of lower forms of life. The stability of the latter to such radiation is extremely high and under terrestrial experimental conditions certain microorganisms can withstand radiation doses considerably higher than those which are encountered in cosmic space without an appreciable fraction of the cells being killed.

Undoubtedly, the picture is completely different in the case of ultraviolet radiation. Among terrestrial microorganisms there are no species which can withstand long exposure to the effect of ultraviolet radiation in doses comparable to those which they would be subjected to in cosmic space. The protection provided by carotinoids and other pigments which might render pigmented bacteria more resistant to the effects of ultraviolet radiation is clearly inadequate. However, by adhering to some material the bacterial cell may become less vulnerable to the action of ultraviolet radiation. Relatively thin layers of matter of which micro-meteorites are composed may fully protect the cell from the lethal effect of ultraviolet radiation. Therefore, there is no reason to assume that existing radiation in space excludes the possibility of maintaining the viability of bacteria. The final solution of this problem will only be obtained by experiments carried out in space.

In accordance with present-day concepts, the most serious difficulty arises when there is no water on a planet. However, our information on the absence of water refers only to the atmosphere and the surface layer of the planet. The possibility cannot be ruled out that at a certain depth water may exist in very small quantities. To take a known analogy, it is possible to refer to the existence of xerophilic microorganisms, particularly actinomycetes and fungi which, although small, do develop in the sands of deserts. It is possible to assume the existence of extraterrestrial forms of life in which the solvent for a number of substances will

not be water but other chemical compounds, for instance, glycol. However, it is more logical to assume that extraterrestrial microorganisms possess a much higher adaptability to life in surroundings containing only very little water than do terrestrial organisms.

Absence of free oxygen does not necessarily prevent development of life; elucidation of the evolution of various physiological functions under conditions of anaerobiosis could throw light on the evolution of terrestrial anaerobic microorganisms. The nature of the physiology of anaerobic microbes may vary greatly. It is possible for anaerobic forms to develop which can oxidize a number of substances, at the expense of bound oxygen. Anaerobic forms do exist which possess a hydrogenase system and which are able to activate hydrogen. These forms include, particularly, bacteria which can fix nitrogen. There is no doubt at all that nitrogen is in the atmosphere of some planets. The existence of hydrocarbons in certain meteorites as well as the detection of hydrocarbons in the atmosphere of planets focus attention on the possibility of the existence in space of microorganisms utilizing the hydrocarbons. From this point of view, the existence on Earth of anaerobic strains of Clostridia, which decompose methane and form acetic acid, as well as bacteria which can form the same acid from carbonic acid and hydrogen is of particular interest. Formation of acetic acid by this means enables an explanation to be put forward for the biosynthesis of amino acids since glutamic acid and other amino acids can easily be obtained from acetic acid.

There is no reason to offer an explanation for the existence of oxygen in the atmosphere of other planets other than that which is offered for its presence in the atmosphere of the Earth. Oxygen is generated in the atmosphere by the action of photosynthetic organisms. Apparently, this was first linked with the appearance of microscopic water plants but this stage of evolution occurred considerably later than the formation of heterotrophic bacteria.

One of the planets on which life will most probably be detected is Mars. This view is supported by the investigations of Sinton, who used a 200-inch telescope and by means of infrared spectroscopy observed absorption bands at a wavelength approaching $3.5\mu$. He discovered a similar absorption band in the spectrum of lichens and the alga Cladophora. Seasonal changes on Mars, reported by Dolfuss, also seem to indicate the existence of life on this planet.

Thus, it is theoretically possible to predict the presence in space of microorganisms with a great variety of physiological properties since the ecological conditions existing, for instance, on Mars, fully provide for the development of single-cell forms of life possessing a variety of metabolic abilities. It is most likely that bacteria exist in space which can denitrify, desulfurate, fix nitrogen, photosynthesize and oxidize hydrocarbons.

We understand by single-cell bodies - microorganisms - since in the absence of these it is difficult to visualize life under any conditions. Apart from this, microorganisms can be detected more easily than other forms of life because they are very small in size, widely scattered and, particularly, because their presence can be detected by a great variety of methods.

Every experimental biologist will obviously be most interested in the principles on which methods of detecting various forms of life in space and on other planets can be based. In the first place, it should be pointed out that development of these methods can be started only on the basis of those features of metabolism, structure and state which are characteristic for single-cell bodies on Earth. Only when negative results are obtained from such an approach will it be appropriate to apply new methods based on the features of the ecological conditions found to exist on one planet or the other.

The study of phosphorus metabolism has helped greatly in the development of modern biochemistry and physiology. In accordance with present-day views, it is difficult to imagine the existence of primitive forms of life that do not contain phosphorus compounds. Therefore, it is desirable to develop sensitive methods of detecting nucleic acids and other phosphorus-containing organic compounds which could be applied in space investigations.

Cells can be detected by various methods. The most attractive is the direct microscopic investigation of the soil or water and transmission of visual pictures of these to Earth. Such microscopy can be combined with application of luminescent dyes, certain microchemical reactions, etc. However, an automatic instrument which could carry out these tasks would be relatively complex and heavy. Some investigators propose that good nutrient media should be exposed to cosmic dust or to the atmosphere of planets. If colonies were to grow on these nutrient media, their appearance could be observed by a special, small microscope, which could be transmitted to Earth.

Intensive multiplication of microorganisms in clear liquids should produce clouding and nephelometric measurements should also serve as an indication of the development of microorganisms. However, it is necessary to be certain that the clouding of the medium is not due to other purely chemical reasons. If multiplication of bacteria in the liquid medium leads to the evolution of gas the existence of microorganisms could also be deduced from manometric measurements. It is also possible to use liquid media which contain labelled carbon compounds which decompose easily. Subsequent determination of the labelled carbon in the formed carbon dioxide will enable an estimation of the speed of development of microorganisms in this medium to be made as well as utilization of the substance containing labelled carbon. The more that is known of the physical and

chemical conditions existing on a given planet, the easier it will be to devise methods for detecting life on it.

In addition to designing various instruments which should operate automatically and serve the purpose of detecting life on the planets, it should also be possible to take specimens of soil, dust, atmosphere or water from the planets and, after bringing them to Earth, search for forms of life in them by means of a great variety of methods. When taking samples and investigating them in laboratories, as well as placing automatic instruments on the surface of the planet to detect life, the extremely critical and difficult requirement to be fulfilled is the prevention of contamination with terrestrial organisms. There is the inherent danger that among specimens taken in the stratosphere or in space by means of stratostats, balloons or rockets for the purpose of subsequent microbiological investigations are organisms which were trapped on Earth by the equipment. As an illustration of the difficulties involved in taking sterile specimens, the case of soil specimens taken from regions of permanent ice can be mentioned. About 20 years ago, a communication was published which showed that after sowing specimens of soil taken from a permafrost region in Siberia, living water plants, protozoa, and microorganisms were detected. The authors believed that these organisms revived after being in an anabiotic state for thousands of years. At the Institute of Microbiology of the Academy of Sciences of the USSR a technique was developed for taking soil samples in a region of ice by means of an electric saw or electric drill. This method excluded the possibility of living organisms falling onto the surface layers of the ground. The specimens taken were transported immediately to the Institute and investigated. In spite of using a variety of growth media, no forms of life were detected in any of the experiments and, consequently, the earlier published findings were due to using imperfect methods of sampling the soil in the permafrost region. Research workers analyzing specimens brought back from space will face exactly the same difficulties. Unfortunately, detection of [Translator's note: contamination with] terrestrial organisms in such specimens is very much easier than one would like it to be. It is necessary to develop a detailed technique of investigation of such specimens.

Such an exacting and cautious attitude is also made necessary by the fact that forms of life brought back with the specimens may be capable of multiplying on Earth. If these forms were to begin to behave like terrestrial microflora and be pathogenic for plants, animals or man, we would encounter a situation similar to that which has been described by Wells in his novel a long time ago.

Investigation in the field of space biology is only beginning. However, undoubtedly space biology will develop at the same pace as the science and technology associated with the design of new spaceships develop. It is at present difficult to forecast when the discovery of new forms of

life in space will be made. There is no doubt that these discoveries will be of extreme importance for the life sciences and the possibility cannot be ruled out that, among the newly discovered microorganisms, forms will be found which are characterized by possessing peculiar metabolic abilities and that these forms may have practical application.

SUMMARY

11671

The author briefly reviews past and present origin-of-life theories and the possibility of interplanetary transport of life forms by means of radiation pressure. It is simpler in principle to suppose that life forms on other planets will resemble terrestrial unicellular organisms, rather than to postulate the existence of types of metabolism not based on water and carbon. Such organisms can withstand liquid helium temperatures but -10° C appears to be the lower limit at which multiplication has been observed. Some thermophilic bacteria can withstand boiling for 4-5 days, and the author postulates that organisms could have become adapted to temperatures up to 150° C on other planets. Many lower terrestrial forms are sufficiently radio-resistant to withstand the effects of cosmic radiation but resistance to ultraviolet radiation is much lower. The absence of oxygen is, in this respect, also immaterial as many terrestrial organisms can grow under anaerobic conditions. The author discusses in conclusion various methods proposed for automatically recording the existence of life forms from a vehicle which has landed on a planet.

Author

NOTE: This is the text of a report at the Conference on Space Biology, held in Moscow (August 17, 1961).

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# ON THE PROBLEM OF SUPPLYING THE CREWS OF SPACESHIPS WITH ANIMAL FOODSTUFFS

K. S. Akhlebininskiy, V. P. Bychkov, I. A. Il'ina,
Yu. I. Kondrat'yev and A. S. Ushakov

At present, most investigators working on problems relating to feeding man in space consider that, for short space flights (up to six months), food provision for the crew can be best ensured by carrying adequate food reserves. However, due to the limited weight and space of the spaceship capsule, the food supply for longer flights (lasting several years) can only be ensured by creating a closed cycle inside the capsule (Sisakyan, 1961; Tisher, 1960a, 1960b; Briggs, 1960, Clark, 1958).

In the closed-cycle system the problem of providing a reliable food supply system is the most difficult and therefore the most urgent which has to be resolved before men can embark on long space flights. However, creation of a completely closed cycle on a physicochemical basis (decomposition of the waste products to the molecular atomic level, organic synthesis of the initial products and repeated utilization of these) is difficult to achieve with our present state of knowledge (Clark, 1959).

At present, the most easily achievable system is the creation of a closed cycle on the basis of single-cell water plants, zooplankton, higher-order plants, animals and man. Single-cell water plants and higher-order plants can be utilized for biological regeneration of air, purification of water and as food for animals and man.

The food ration of an astronaut should be fully satisfactory as regards quantity and quality. The total calorific value of the ration should average 3000 kcal/day/man. The calorific value should be distributed as follows: protein 14 percent of the ration, fats 25 percent and carbohydrates 61 percent. The weight distribution should be: protein 104 g; fats 80 g and carbohydrates 447 g. At least 40-60 percent of the protein should be of animal origin and at least 60 percent of the fats should be of animal origin. The daily requirements of the astronaut in animal proteins and fats can be satisfied by inclusion into the food system of various animals - from very simple to those of a higher-order.

At present the most suitable are those types of higher animals which have been sufficiently well studied from the zoological, food and physiological points of view, the composition of which is the most economic - for instance, domestic birds (hens, ducks), rabbits, etc. From literary data and calculations made by the authors the preliminary conclusion is made that the most suitable food for the given conditions are chickens.

A tentative calculation was made of the required number of chickens to take on board. Chickens provide high-quality, tasty meat and eggs, a high weight increase per unit of food consumed, a high percentage (62) of meat yield and they grow quickly. Hens can be fed by single-cell water plants, leftover food scraps, waste from killed birds (offal, processed bones, feathers, eggshells).

Methods of forced machine feeding can be developed for hens, which may be important under conditions of weightlessness and when using unusual foodstuffs. They can live in a limited space (in cages).

Furthermore, hens can be bred by incubation of the eggs. This is important since in the case of the hens dying-off, the stock can be replaced provided there is a certain reserve of eggs.

The requirements of animal protein per person per day can be satisfied by including in the ration 280 g of assimilable chicken meat and 2 eggs.

To obtain this quantity of meat and eggs per person, per day, it is necessary to have a permanent stock of the following size: 10 grown hens, 2 cockerels, 4 four-month old chicks, 17 three-month old chicks, 17 two-months old chicks, 17 one-month old chicks and 17 day-old chicks. In addition, two reserve chickens have to be carried. The stud cockerels should be replaced every three months. At other times, hens should be used for meat. The meat from the spare hens is not taken into consideration in the overall balance sheet since these are intended to replace diseased and deceased chickens.

Two mature chickens and 13 three-month old chickens should be killed every month. The data on the monthly quantity of meat obtained from killed chickens are given in Table 1.

Table 1. Data on the Quantity of Meat (Including the Heart and Liver) Obtained from a Killed Bird Per Month

| | Average weight per bird kg | Number of killed birds | Meat-yield per bird, kg | Meat-yield of all the birds killed, kg |
|---|---|---|---|---|
| Hens | 1.7 | 2 | 1.05 | 2.1 |
| Young chickens | 0.9 | 13 | 0.558 | 7.25 |
| | | | Total | 9.35 |

In the case of continuous operation of the system, there will be no necessity to replace the stock of chickens and this will permit obtaining additionally 3.400 kg of live weight of meat per month, with a meat yield of 2.100 kg.

Of the 9.35 kg meat obtained, 90 percent or 8.415 kg of meat is assimilable. The 8.415 kg of meat contains 18.5 percent protein, i.e. 1.556 kg of protein, which corresponds to 52 g protein per man per day. The quantity of protein obtained from 2 eggs (average weight 50 g) is 13 g and the assimilable quantity of protein is 95 percent, i.e. 12.3 g.

Consequently, the quantity of assimilable proteins obtained from products of animal origin will equal 64.3 g/day (the required standard being 62.4). The same quantity of chicken meat contains about 17 percent or 14.31 kg fat, which amounts to about 48 g of fat per day.

The quantity of fat obtained from two eggs (average weight 50 g) is 11.6 g, of which 95 percent, i.e. 11.0 g, is assimilable. Consequently, the quantity of fats obtained from products of animal origin will equal about 59 g (the standard being 48.0 g).

Thus, one man will receive 64.3 x 4.1 = 264.0 kcal per day of protein of animal origin and 59.0 x 9.3 = 549.0 kcal of fats of animal origin and 264.0 + 549.0 = 813.0 kcal from the products of animal origin, which is over 27 percent of the daily calorie ration.

For feeding this stock of birds, a ration is proposed which would include single-cell water plants, parts of higher-order plants, which are not used in the human food ration, wastes from the food of the personnel, eggshells, etc.

For planning and providing the ration made up of the above enumerated substances, additional experimental data are required. Due to the absence of the necessary data all the calculations are based on the makeup of the birds under terrestrial conditions. It can be assumed that the given data will not differ significantly from data obtained under the particular conditions mentioned above.

Table 2 gives data on the quantity of foodstuffs required by birds of different ages (in food units).

The daily water requirement of the entire flock is given in Table 3.

The doses of ultraviolet radiation required for normal development of the birds are given in Table 4.

The recommended number of hours of ultraviolet irradiation (in terms of terrestrial days): hens - from 6 to 9 hours, from 12 to 15 hours, from 17 to 20 hours; chicks - up to three times per day.

Table 2. Data on the Quantity of Foodstuffs Required Per Day by Birds of Different Ages (in Food Units)

| Age of bird | Total No. of birds | Average weight per bird, g | No. of food units required per bird, g | No. of food units required per cockerel, g | Total No. of food units for birds of different age groups, g |
|---|---|---|---|---|---|
| 1 day | 17 | 35 | 5 | | 85 |
| 1 month | 17 | 230 | 34.9 | | 593.3 |
| 2 months | 17 | 550 | 85.3 | 95.02 | 1469.54 |
| 3 months | 17 | 900 | 105 | 117.6 | 1810.2 |
| 4 months | 4 | 1200 | 117.3 | 131.38 | 497.36 |
| 5 months | 4 | 1500 | 128.9 | 144.39 | 546.68 |
| Mature | 12 | 1700 | 129.5 | 145.04 | 1585.08 |

Total: 6587.06 food units (g) ≅ ≅ 6.5 food units (kg)

Illumination of the sections housing the mature birds - artificial illumination of 3 to 5 $W/m^2$ of area for 12 to 13 hours per day.

Eggs intended for incubation must be stored in adequately ventilated storage space at a temperature of $+8^{\circ}$ C with a relative humidity of 65 to 70 percent.

Table 3. Daily Water Requirements of the Entire Flock of Birds

| Age of bird | No. of birds | Daily requirements, ml | Total water quantity required, ml |
|---|---|---|---|
| 1 day | 17 | 32 | 544 |
| 1 month | 17 | 45 | 765 |
| 2 months | 17 | 150 | 2550 |
| 3 months | 17 | 180 | 3060 |
| 4 months | 4 | 250 | 1000 |
| 5 months | 4 | 250 | 1000 |
| Mature | 12 | 300 | 3600 |
| | Total | | 12.5 liters |

Note: The given figures include drinking water, water for preparing food and for hygienic purposes.

Table 4. Ultraviolet-radiation Dose Required for Normal Development of Birds

| | Radiation dose, mer*/h/$m^2$ | Erythemal "irradiatedness" mer/$m^2$ | Daily duration of radiation, hrs |
|---|---|---|---|
| Hens | 180 | 18-22 | 9 |
| Chicks | From 20 to 140 with gradual increase of radiation dose | 16-20 | 0.5-0.8** |

*Presumably rem/h/$m^2$.
**Duration of radiation is gradually increased.

The temperature of the incubator should be 37.5° C, the relative humidity 54 to 55 percent. The relative humidity in the discharge section should be 75-80 percent. Three sections should be equipped for raising young chicks. In the first section, the chicks would be raised from the age of 1 to 10 days, keeping them at 30-26° C and in a relative humidity of 65-75 percent; in the second section, the chicks would be kept from the 11th to the 20th day at 25-23° C in a relative humidity of 65-75 percent. In the third section, the chicks would be kept from the 21st to the 30th day at 23-20° C in a relative humidity of 50-70 percent. For mature hens a relative air humidity of 50-55 percent is recommended.

The daily gas exchange rate is entered in Table 5.

Thus, the flock of chickens on board for providing the animal-food supply of one man will require: 2283.06 liters oxygen; 6.5 food units (kg); 12.5 liters water. They will generate 2060.10 liters of carbon dioxide.

The weight of the entire flock of birds is 60-61 kg and for housing it, a space of about 1.8 $m^3$ is required.

In carrying out the calculations, the authors took into consideration the difficulty of creating on board the spaceship optimal conditions of raising chickens and therefore an average live weight of both mature hens and chicks was assumed (weight of mature hen - 1.7 kg; weight of three-month old chick - 0.9 kg).

Furthermore, the authors assumed that 60 percent of the human protein ration would be animal protein.

If the living conditions for the chickens in the capsule are optimal and a good species is selected, for instance, general-use "Kuchinska" (weight of hen 3.0-3.6 kg, weight of a three-month old broiler 1.5 kg) and assuming that, of the total quantity of protein for the crew, 40 percent will be of animal protein, the size of the bird flock may be reduced accordingly (by a factor exceeding 2); this will obviously also permit reducing the required quantity of food, water and other supplies necessary for maintaining the birds.

SUMMARY

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The authors consider that the most realistic way to provide spaceship crews with food of animal origin is to carry and raise in these

Table 5. Calculated Daily Gas Exchange of the Bird Flock

| Age of bird | Wt. alive, g | No. of chicks | Wt. of entire group, g | Generation of $CO_2$, lit/kg/24 hr | Generation of $CO_2$ of entire group, l | Absorption of $O_2$, lit/kg/24 hr | Absorption of $O_2$ by whole live-stock, l |
|---|---|---|---|---|---|---|---|
| 1 day | 35 | 17 | 595 | 38.00 | 22.61 | 47.70 | 28.38 |
| 1 month | 230 | 17 | 3,910 | 52.20 | 204.10 | 56.90 | 222.48 |
| 2 months | 550 | 17 | 9,350 | 45.00 | 420.75 | 49.30 | 460.96 |
| 3 months (80 days)* | 900 | 17 | 15,300 | 41.61 | 636.63 | 45.39 | 694.46 |
| 4 months | 1,200 | 4** | 4,800 | 31.97 | 153.45 | 34.60 | 166.08 |
| 5 months | 1,500 | 4** | 6,000 | 33.72 | 202.32 | 36.51 | 219.06 |
| Mature hens including 2 cockerels | 1,700 | 12 | 20,400 | 20.60 | 420.24 | 24.10 | 491.64 |
| Total | | | 60,355 | | 2,060.10 | | 2,283.06 |

*The authors have no data on the gas-exchange of 90-day old chicks.
**Of these, two are spare.

spaceships higher-order animals which have been thoroughly studied from the zoological, physiological and nutritional point of view, for instance, poultry, rabbits, etc. In particular, they suggest as being very economical chickens which can be fed on algae and food wastes, including chicken entrails, and only require a small space.

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# PART II

# BIOLOGICAL EXPERIMENTS ON SPACESHIPS AND SATELLITES

## BIOCHEMICAL INVESTIGATIONS OF THE BLOOD AND URINE OF ANIMALS THAT FLEW IN SATELLITES

A. A. Gyurdzhian, N. N. Demin, L. T. Tutochkina,

M. S. Uspenskaya, T. A. Federova

In order to discover the biological effects of cosmic radiation and other flight factors, a special series of biochemical indices was worked out which changed considerably during "stress-states" and under the influence of ionizing radiations. These indices included determination of the protein composition and the non-specific cholinesterase activity of the blood serum as well as the corticosteroids and the intermediates of nucleic acid metabolism in urine (Gyurdzhian, Demin, Korneyeva et al., 1961).

### Experimental Animals, Experimental Conditions and Methods of Investigation

The animals used for these investigations were mongrel dogs of 3 to 6 years old. In some of the experiments white rats and mice of the line $C_{57}$ were used.

The dogs were examined before and after the flights on the second Soviet spaceships which made 17 orbits around the Earth (Belka, Strelka), on the fourth and fifth spaceships, which made one orbit around the Earth (Chernushka, Zvezdochka).

To discover the influence of individual flight factors (acceleration and vibration), special experiments were carried out using a centrifuge and a vibration test-stand in which the dogs were subjected to the effect of accelerations of 1-5 min duration and a maximum of 6-9 g, or to the effect of vibrations of 70 cps with an amplitude of 0.4 mm and durations of 5-12 min. Thirteen vibration tests were made and 33 centrifuge tests. In these experiments 18 dogs were used, five of which had flown earlier in 1958 and 1959 in high-altitude rockets; (Otvazhnaya flew five times, Damka - three times, Snezhinka and Pal'ma - twice, Zhemchuzhnaya - once). Four dogs who did not fly were trained according to the same program as those who did fly. The remaining nine were

subjected to a more complex series of investigations: in addition to repeated centrifuging and vibration, they were put into a sealed capsule with air regeneration, special conditions of feeding, water supply and waste removal and were subjected to the effects of elevated and low temperatures.

Blood samples of 6-7 ml were taken from the femoral vein on an empty stomach. Daily urine specimens were taken when the dogs were put into an "exchange" cage or a special room where they were under constant observation and periodically they were made to walk while wearing a rubber waste-removal device (Gazenko, Gyurdzhian, Zakhar'yev, 1961). For determining the corticosteroids and the deoxycytidine in the urine, thymol was added as an antiseptic; for determining the Dische-positive substances the urine specimen was precipitated with 96 percent alcohol in the ratio of 1:1.

The total protein concentration in the blood serum was determined refractometrically; the ratio of the protein fractions was determined by paper electrophoresis; the concentration of serum mucoids by the method of Wintzler; the nonspecific cholinesterase activity of the serum by the method of Zubkova and Pravdich-Neminskaya; the content of free and combined 21-oxy-20-ketocorticosteroids in the urine according to the method of Silver and Porter; and deoxycytidine and Dische-positive substances in the urine according to the reaction of Dische as modified by Shtumpf and using paper chromatography according to Parzizek, Arient, Dienstbier and Skoda. The methods of investigation have been described in greater detail in an earlier paper (Gyurdzhian et al., 1961).

## Results

Laboratory Tests. In Figure 1 data are given which characterize the protein composition of the blood serum of the dogs examined between October, 1959 and September, 1960. The experimental animals were subdivided into four groups. The first group included 20 healthy dogs which were not subjected to any experiments. The second group comprised five dogs which had previously flown in high-altitude rockets, the third group comprised four dogs who had not flown. The dogs of the second and third groups were kept under identical conditions and subjected to repeated centrifuging and vibration tests. The fourth group comprised nine dogs who had not flown but were subjected to a more complex series of investigations as mentioned above. Each dog was examined some five to ten times during the year before the acceleration or vibration tests.

The obtained data indicate that during the period of intensive investigation the protein composition of the blood serum of the dogs fluctuated strongly and in a number of cases differed greatly from the standard value. Compared to the dogs of the first group, which were not

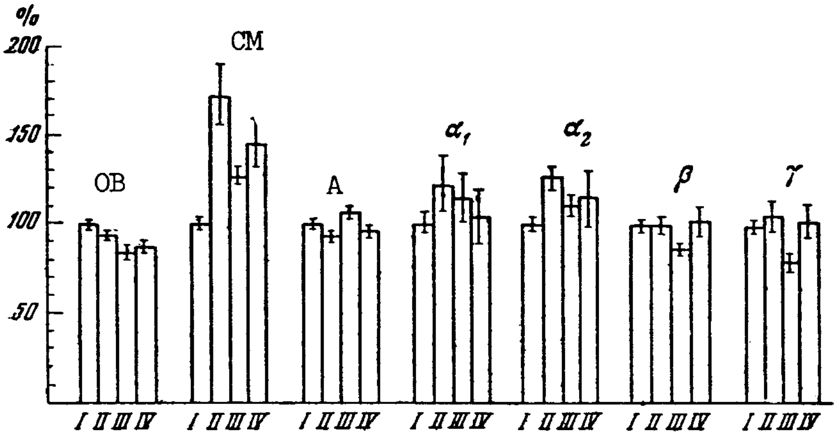


Figure 1. Protein Composition of the Blood Serum of Dogs During the Laboratory Investigations (Background from October, 1959 through September, 1960). I - Dogs not subjected to experiments; II - Dogs which had flown (Damka, Otvazhnaya, Zhemchuzhnaya, Pal'ma, Snezinka); III - Dogs which had not flown (Neva, Volga, Malek, Rechka); IV - Dogs which had not flown but were subjected to more complex investigations (Marsianka, Lisichka, Gil'da, Il'va, Sil'va, Laska, Pchelka, Tsikada). The results are expressed as a percent of the average indices of the first group. The vertical lines indicate the standard deviations: CM - serum mucoids; OB - total protein concentration; A - albumins; $\alpha_1$ - $\alpha_2$ - $\beta$ - $\gamma$ - globulins.

subjected to any experiments, the relative amounts of $\alpha_1$ and $\alpha_2$-globulins were high, while the relative amounts of albumins and the total protein concentration were low. The most significant, statistically reliable, changes were detected in the fraction of serum mucoids, the concentration of which increased sharply.

The detected changes are characteristic for dystrophic states, in fact, some of the dogs showed a clinically pronounced muscular dystrophy. The dogs of the second group, who had flown in the past and who were subjected to the experimental conditions over a long period, showed more pronounced dystrophic changes than the dogs of the third group who had not flown. As regards the cholinesterase activity of the blood serum, no difference could be established between the two groups.

Directly after a single centrifuge or vibration experiment, slight changes of a short duration (1-6 days) in biochemical indices of a different type were detected. Thus, the tendency for a decrease in the concentration of serum mucoids to occur was more pronounced in the group of dogs who had not flown (Figure 2). The most characteristic change, however, was the clear and statistically reliable increase of cholinesterase activity reaching a maximum after 1-3 days (Figure 3).

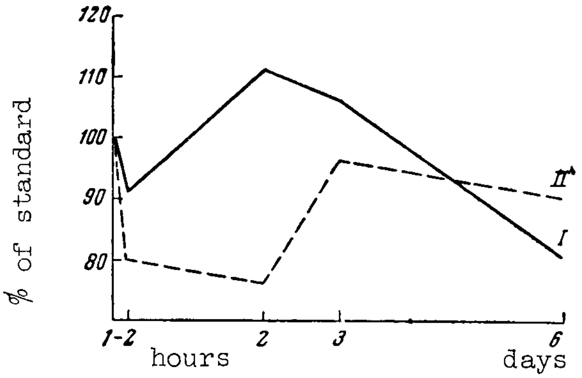


Figure 2. Serum Mucoid Changes Caused by Acceleration Effects. I - Dogs who had flown (Damka, Otvazhnaya, Zhemchuzhnaya, Pal'ma, Snezinka); II - Dogs who had not flown (Neva, Volga, Malek, Rechka).

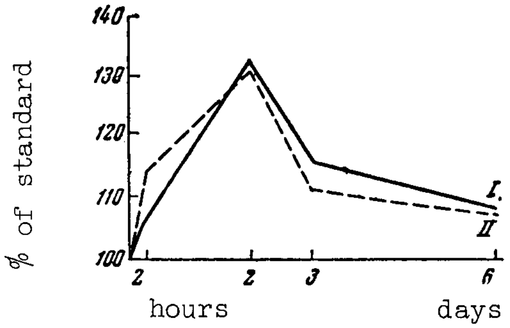


Figure 3. Cholinesterase Activity in the Blood Serum of Dogs Subjected to Acceleration Effects. I - Dogs who had flown; II - Dogs who had not flown (Neva, Volga, Marsianka, Lisichka, Gil'da, Il'va, Strelka Belka, Linda, Sil'va, Laska, Pchelka).

Similar nonspecific changes were observed in compensated forms of various diseases and when weak toxic agents were administered. These changes suggest activation of certain liver functions and of some other organ systems of the body.

Thus, in the case of a single centrifuging on the one hand, and in the case of the action of an entire range of investigations on the other hand, opposite shifts of some biochemical indices were established. The described case has an analogy in the known physiological phenomenon in which, depending on the duration of the action and the time interval between the action, and when recovery takes place exhausting loads may lead to development of two opposite states - activation or exhaustion.

Changes After Flights. The second spaceship carried two dogs: Belka and Strelka. Both flew for the first time. According to the biochemical data, both dogs were in good health prior to the flight.

After returning from the flight an appreciable increase in the concentration of serum mucoids and $\alpha_2$-globulins was observed after 2-6 days (see Table). The total protein concentration also showed a tendency to increase. The cholinesterase activity of the blood fluctuated towards a lower value. These shifts stopped some 13-23 days after the end of the flight.

During the same period of time, a similar increase in the total protein concentration and the concentration of serum mucoids was observed in Strelka but the relative content of $\alpha_2$ globulins had not changed. The cholinesterase activity decreased two days after returning from the flight.

Thus, after the flight on the second spaceship only a slightly pronounced "stress" type reaction was observed in Belka and Strelka, which consisted of a short duration increase in the concentration of serum mucoids and $\alpha_2$-globulins (in Belka) as well as a decrease in the cholinesterase activity. Then all the indices returned to normal and during a long period of observation (over a year) did not exceed the normal limits.

In mice which flew on the second spaceship changes in the protein composition of the blood serum took place 5 days after returning: in particular a decrease of the relative content of albumins and an increase of all the globulin fractions. However, only nine days after returning from the flight the difference in the amounts of the protein fractions between the mice which flew and the controls was insignificant.

Changes in the Biochemical Indices of Belka and Strelka After the Flight

| Name | Time of analysis, days after flight | OB, g % | CM, mg % thyroxin | Relative content of protein fractions, g % | | | | | ChEA | DOTs in the daily urine, % of normal |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Albumins | | | Globulins | | | |
| | | | | | $\alpha_1$ | $\alpha_2$ | $\beta$ | $\gamma$ | | |
| Belka | Before flight* | 6.3 | 2.8 | 60.8 | 5.3 | 9.9 | 11.6 | 12.0 | 0.84 | 100 |
| | 2 | 6.4 | 3.7 | 57.5 | 4.5 | 12.1 | 14.1 | 11.6 | 0.78 | |
| | 6-7 | 7.0 | 4.4 | 58.9 | 4.8 | 11.3 | 15.0 | 10.0 | 0.84 | 77.7 |
| | 13 | 6.5 | 3.0 | 59.3 | 4.7 | 9.5 | 14.7 | 11.7 | 0.77 | 59.8 |
| | 23-25 | 5.8 | 2.8 | 58.8 | 5.2 | 9.6 | 14.8 | 11.6 | 0.87 | 45.8 |
| Strelka | Before flight | 6.4 | 2.1 | 60.5 | 3.8 | 10.9** | 12.7 | 12.1 | 0.78 | 100 |
| | 2 | 6.4 | 2.8 | 62.9 | 3.6 | 8.5 | 11.5 | 13.5 | 0.66 | |
| | 6-7 | 7.5 | 3.6 | 60.2 | 5.2 | 8.8 | 13.7 | 12.0 | 0.91 | 129.9 |
| | 13 | 7.1 | 2.8 | 57.1 | 4.7 | 8.9 | 14.8 | 14.6 | 0.87 | 139.5 |
| | 23-25 | 6.4 | 2.0 | 57.4 | 4.5 | 8.2 | 14.3 | 15.6 | 0.91 | 129.9 |

OB - total protein concentration; CM - serum mucoids; ChEA - cholinesterase activity expressed in mg of benzoylcholine, destroyed by 0.1 ml of blood serum per hour; DOTs - deoxycytidine.

* Specimens taken 2-3 days before launching.

** Value of the fractions of $\alpha_1$-globulins, these were apparently too high due to the hemolysis of the blood serum.

No regular changes of the diuresis and the specific gravity of the urine were detected in Belka and Strelka after the flight. The content of Dische-positive substances of deoxycytidine in the urine changed equivocally: during the seven days after returning from the flight a considerable drop in the deoxycytidine was observed in Belka and a slight increase in Strelka.

In the two rats which flew in the second spaceship, the content of Dische-positive substances 6, 7, 12, 13 and 14 days after return did not differ from that of the control animals.

In the mice, the content of Dische-positive substances in the urine was two times lower than in the controls; then for 6, 7, and 11 days no changes were observed in the contents of these substances in the urine.

Thus, the obtained data indicate that no permanent breakdown occurs in the metabolism of the nucleic acids in the bodies of animals who fly (Gyurdzhian et al., 1961).

The concentration of free corticosteroids in the urine of Belka and Strelka 7-27 days after the flight was below normal (0.01-0.03 mg per 24 hours as compared to the normal 0.02-0.26 mg).

In the dogs Chernushka and Zvezdochka, who flew on the fourth and fifth spaceships, no disturbances in their metabolism could be observed when compared with the control dog Kometa, who was subjected to the same training and, during the entire period of investigation, except for the actual flight, was kept under exactly the same conditions (including transportation to the launching pad and staying there).

From February to May, 1961 slight but regular changes occurred in all three dogs: a drop in the release of free 21-oxy-20-ketocorticosteroid in the urine (Figure 4,b), a drop in the concentration of the serum mucoids (Figure 5,a), an increase in the cholinesterase activity of the blood (Figure 5,b). The relative content of albumins was at the upper limit of the normal value.

The deoxycytidine content in the urine of dogs which had flown varied within the limits observed in the control dogs (Figure 4,a).

The described shifts in Chernushka and Zvezdochka were within the normal physiological limits and apparently were caused by seasonal factors (spring), changes in climatic conditions, and environmental conditions (travel to the launching pad, stoppage of training and tests).

The selected series of biochemical indices permit differentiating between:

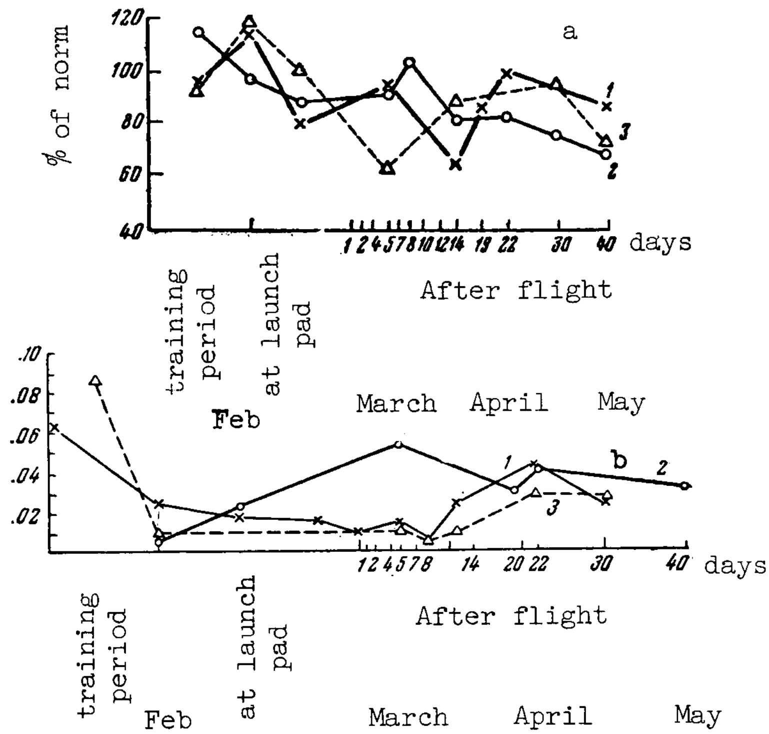


Figure 4. Release of Deoxycytidine (a) and 21-oxy-20-ketocorticosteroids (b) in the daily urine specimen of the dogs who had flown on the fourth and fifth satellites. Along the ordinates - amount of deoxycytidine as percent of normal, and 21-oxy-20-ketocorticosteroids in the urine in mg; along the abscissa - duration of the investigations in days. 1 - Chernushka; 2 - Zvezdochka; 3 - control dog Kometa.

1. Activation of the function of some organs and systems of the experimental dogs (in the case of single action of moderate accelerations or vibrations);

2. Dystrophic state (under the influence of particular experiments, training and observation);

3. "Stress" type reaction in the case of prolonged orbital flights (of the order of a day) which was characterized by a reversible depression of functions which subsequently recovered. This reaction could not be established after short duration (single orbit) flights.

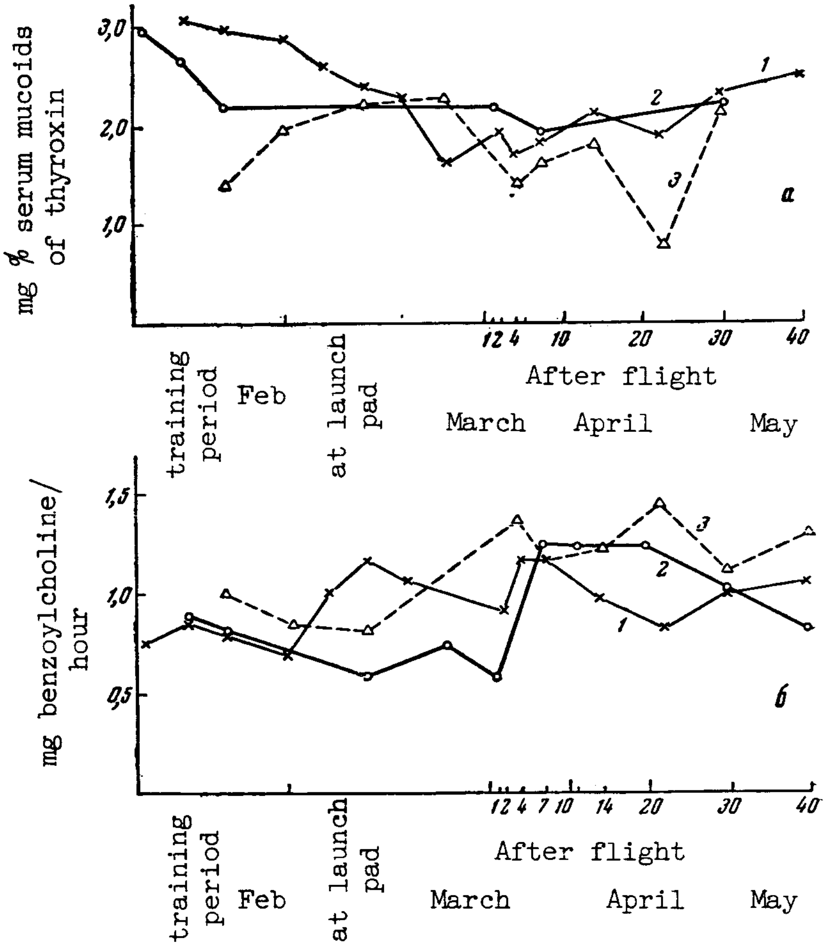


Figure 5. Concentration of serum mucoids (a) and non-specific cholinesterase activity of the blood serum (b) in dogs who flew on the fourth and fifth spaceships.

On the basis of the investigations carried out, it can be concluded that a single flight in space under conditions pertaining to the second, fourth and fifth spaceships does not cause irreversible changes in the metabolism. The dynamics of the changes in the investigated indices after flight was more characteristic of "stress" reactions than of radiation injury.

The authors express acknowledgment to V. V. Antipov, V. G. Vysotskiy, N. V. Korneyeva and M. M. Skurikhinaya who participated in this work.

Summary

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The studies described included: determination of the protein composition of blood and its nonspecific cholinesterase activity, of the amount of 21-oxy-20-ketocorticosteroids and intermediates of nucleic acid metabolism (deoxicytidine and Dische-positive substances) contained in urine.

Dogs were studied under laboratory conditions (exposure to acceleration, vibration and other factors) and on board the Soviet satellites 2, 4 and 5. After a single period of acceleration or vibration under conditions similar to those pertaining to flights, relatively short-term changes were found to exist, the changes indicating an activation of the functions of the liver and other systems, i.e., an increase in the nonspecific cholinesterase activity and a decrease in the concentration of serum mucoids in blood.

Due to prolonged training, tests, rocket flights and examinations, some dogs developed a state of dystrophia characterized by a stable rise of serum mucoids and $\alpha_2$-globulin concentration in blood and by a reduction of the total amount of protein at the expense of the albumin fraction.

After a 24-hour flight, Belka and Strelka showed transient biochemical changes that are characteristic of reversible and moderate stress-reactions, i.e., a short duration increase in $\alpha_2$-globulin and serum mucoids (2-6 days after the flight). No changes were detected in the metabolism of the dogs Chernushka and Zvezdochka whose flight only lasted for 90 min.

It is concluded that the biochemical changes observed after the flight are more likely to be due to stress reactions than to ionizing radiation.

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# CHANGES IN CERTAIN MORPHOLOGICAL AND BIOCHEMICAL INDICES OF THE PERIPHERAL BLOOD OF ANIMALS AFTER ROCKET FLIGHTS

I. I. Kas'yan, Ye. M. Yuganov, T. S. L'vova

The aim of the investigation was to study the morphological and biochemical composition of the peripheral blood of animals after rocket flights.

Investigation of the morphological indices of the peripheral blood of animals under conditions of space flight in rockets is of major scientific interest. So far only an insignificant number of papers have been published by Soviet and non-Soviet authors on the morphological composition of the blood of animals which have flown on high-altitude rockets (Galkin et al., 1958; Bugrov et al., 1958). The experiments were carried out on twelve mongrel dogs of both sexes, 3 to 5 years old and weighing 5-7 kg. Altogether 42 investigations were carried out. Most of the animals flew two to three times and some three to five times. Blood samples were taken on the day before a flight, 1.5 to 2 hours after landing and also at later stages. The blood was taken from the vein of a hind paw and an ear. Blood counts were made in a Burker chamber, hemoglobin was determined according to Sully, prothrombin according to Quick, calcium by the method of de Vaard and coagulation by the method of Mass and Magro.

Analysis of the experimental data indicates that after flights in rockets there is an increase in the total number of leucocytes as compared with the initial number.

Data are given below of the count of the number of leucocytes in dogs before and after flight. It can be seen from Table 1 that the increase in the number of leucocytes fluctuated between 1800 and 11,050 cells per $mm^3$.

More pronounced changes were observed in the dog Otvazhnaya which made several rocket flights. In calculating the leucocyte ratio, it was established that neutrophil leucocytosis with a shift of the ratio to the left, and an increase in the quantity of stab nuclear leucocytes occurred. Thereby, the number of unsegmented forms increased from 2 to 30 percent. The number of lymphocytes decreased accordingly. The fluctuation of monocytes and eosinophils was indeterminate.

It can be assumed that the appearance of leucocytosis and of the neutrophil reaction is due to a redistribution of the blood in the body and primarily to the entry of elements of the white cell series into the

Table 1. Change in the Number of Leucocytes

| Name | Number of leucocytes, $mm^3$ | | |
|---|---|---|---|
| | Eve of flight | 1.5 hours after flight | Magnitude of the change |
| Nestraya | 9100 | 18800 | + 9700 |
| Pal'ma | 11400 | 14800 | + 3400 |
| Pal'ma | 15200 | 19600 | + 4400 |
| Snezhinka | 7500 | 9300 | + 1800 |
| Otvazhnaya | 13700 | 23900 | +10200 |
| Otvazhnaya | 7000 | 12500 | + 5500 |
| Otvazhnaya | 7250 | 18300 | +11050 |

blood stream from the depot organs. Such redistribution may be due to the mechanical influence on the body of decelerations during the instant of braking of the nose part of the rocket in the denser layers of the atmosphere. Thereby, it is inadmissible to disregard the importance of "stress" type reactions which may have an appreciable influence on the neuro-reflex mechanisms which control the redistribution of the peripheral blood. Before and after a flight the basic indices of the red blood cells were also measured. The results of the investigations are given in Tables 2 and 3, which show that the red blood cell counts did not deviate from the normal level.

The authors also attempted to elucidate the changes in some biochemical indices of the blood. For this purpose the clotting time of the blood was measured before and after the flight. After the flight the blood clotted more quickly.

The number of thrombocytes, prothrombin and calcium content in the blood were determined from a single blood specimen from each of the three dogs before and after the flights. The data obtained indicate that simultaneously with the reduction in the clotting time of the blood a certain increase of the calcium content, the prothrombin level and a slight, but not always conclusive, increase in the number of thrombocytes occurs.

The authors tend to explain the faster clotting of the blood after rocket flights by an increase of the tone of the sympathetic nervous system due to "stress" type reactions (Frada, Salamone, 1957) or other factors.

Table 2. Dynamics of the Morphological Indices of the Blood of the Dog Otvazhnaya

| Index | 1958 | | | | | 1959 | | 1960 | | | 1961 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Before flight | | | 1.5 hrs after flight | 3 days after flight | Before flight | 1.5 hrs after flight | Before flight | | 1.5 hrs after flight | 1 year after flight | 1 year 3 months after flight |
| Hemoglobin, mg % | 15.3 | 11.8 | 12.5 | 12.3 | 12.2 | 11.7 | 11.4 | 17 | 16.2 | 14.6 | 12.4 | 13.8 |
| Erythrocytes, million per 1 $mm^3$ | 6.25 | 4.50 | 4.55 | 4.15 | 4.55 | 4.25 | 6.43 | 6.43 | 6.43 | 5.90 | 6.49 | 7.56 |
| Leucocytes, thousand per 1 $mm^3$ | 13.6 | 5.7 | 13.7 | 23.9 | 6.55 | 7.0 | 12.5 | 5.9 | 7.25 | 18.3 | 10.2 | 9.2 |
| ROE, mm per hour | -- | -- | -- | -- | -- | 15 | 8 | 6 | 4 | -- | 3 | -- |
| Segments, % | 67 | 82.5 | 79 | 81 | 70 | 77 | 83 | 62 | 47 | 41 | 52 | 49 |
| Slabnuclear, % | 5 | 6 | 6 | 9 | 8 | 2 | 4 | 12 | 15 | 45 | 23 | 18.0 |
| Eosinophils, % | 1 | 0.5 | 1 | 1 | 0.5 | 1 | 1 | 1 | 3 | 2 | 3 | 1.5 |
| Basophils, % | -- | -- | -- | -- | -- | -- | -- | 1 | -- | -- | -- | -- |
| Monocytes, % | 7 | 8 | 5 | 6.5 | 7 | 2 | 2 | 3 | 5 | 5 | 3 | 2.5 |
| Lymphocytes, % | 20 | 3 | 9 | 2.5 | 14.5 | 18 | 10 | 21 | 30 | 7 | 19 | 29 |

Table 3. Dynamics of the Morphological Indices of the Blood of the Dogs Pal'ma, Pestraya and Snezhinka

| Index | Pal'ma 1958 | | | | | | Pestraya 1958 | | | Pestraya 1961 | Snezhinka 1959 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Before flight | 1.5 hrs after flight | Before flight | 1.5 hrs after flight | 2.5 months after flight | 3 months after flight | 2 days before flight | Before flight | 1.5 hrs after flight | 3 years after flight | Before flight | 1.5 hrs after flight |
| Hemoglobin, mg % | 14 | 12.1 | 11.7 | 12.8 | 11.8 | 14 | 14 | 14.3 | 13.8 | 15 | 10.9 | 11 |
| Erythrocytes, million per 1 $mm^3$ | 5.87 | 4.50 | 4.20 | 4.38 | 4.54 | 4.75 | 4.65 | 5.14 | 4.90 | 7.7 | 4.00 | 4.20 |
| Leucocyte, thousand per 1 $mm^3$ | 11.4 | 14.8 | 15.2 | 19.6 | 10.3 | 10.6 | 8.4 | 9.1 | 18.8 | 8.1 | 7.5 | 9.3 |
| Stabnuclear, % | 3 | 2.5 | 5 | 7 | 6 | 5 | 5 | 3.5 | 6 | 3.5 | 1 | 4 |
| Segments, % | 66 | 87.5 | 56 | 64 | 68 | 69 | 73 | 77.5 | 88 | 73 | 75 | 80 |
| Eosinophils, % | | | | 1 | | | 1 | 0.5 | 0.5 | 5.5 | 2 | |
| Basophils, % | | | 1 | | | | | 1 | | | | |
| Monocytes, % | 9 | 2.5 | 3 | 4.5 | 8 | 6.5 | 3 | 9 | 3.5 | 1.5 | 4 | 2 |
| Lymphocytes, % | 22 | 7.5 | 35 | 23.5 | 18 | 19.5 | 18 | 8.5 | 2 | 16.5 | 18 | 14 |

The data given here should be considered preliminary and requiring further study.

Summary

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Blood counts and blood biochemical determinations were carried out on 12 dogs which had undergone 2 to 5 space flights. Blood samples were taken on the eve of the flight, 1-1/2 to 2-1/2 hours after landing and also at later stages. Forty-two investigations were carried out in all. After the flight an increase in the white cell count of 1,800-11,050 cells per $mm^3$ was noted, due mainly to an increase in neutrophils, with a shift to the left of up to 30 percent of unsegmented forms. These changes were ascribed to a redistribution of cells from depot organs under the influence of mechanical forces, although a stress reaction may also have been concerned. No changes were noted in the red cell count, but after the space flight there was a reduction in clotting time, with an increase in the calcium and prothrombin levels and a slight rise in the platelet count.

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# RESULTS OF INVESTIGATIONS OF SOME INDICES OF THE PERIPHERAL VESSELS IN DOGS DURING AND AFTER SPACE FLIGHT

V. V. Yakovlev

The maximum arterial pressure, arterial tone, venous pressure, venous tone and blood flow velocity were studied to determine the various factors which act on the vascular system of animals during flights in rockets.

A plethysmograph universal pressure gauge, an account of which was published by the author in 1958, was used for measuring these indices in a bloodless experiment. The earlier instrument was improved by making it lighter and fitting it with an automatic cup inflator. The plethysmograph clip-unit which recorded oscillations from the ear cavities of the dogs, was made of polyethylene.

The main advantage of this recording method is the almost complete absence of the influence of any movements of the experimental animal on the plethysmograph recording. A semiconductor strain-gauge amplifier was used for transforming the pressure changes of the air into electrical ones.

The changes in the plethysmograph tracing caused by compression and decompression of the air in the ear cavity which are recorded by the inserted cup enables the above enumerated indices of the peripheral blood circulation to be assessed.

## State of the Peripheral Blood Circulation in the Dog Belka After Space Flight

The condition of the peripheral vessels in the dog Belka was investigated six times: before the flight and the first [twice], second, fourth and fifth days after the flight (Table 1).

No changes were observed in the maximum arterial pressure measured in the artery of the ear after the animal returned from the flight when compared with the control observations. In the same way, the venous pressure fluctuated within limits which were characteristic for the given animal prior to its flight into space.

Slight changes were detected in the arterial and venous tone and the blood flow velocity. These three indices of the state of the peripheral vessels are important indicators of the extent of vessel dilatation. A drop in the tone of the arterial and venous vessels indicates that they are somewhat dilated.

Table 1. Data on the State of the Peripheral Blood Circulation of Belka

Before flight

| No. of examinations | Arterial tone | Arterial pressure, mm Hg | Venous pressure, mm Hg | Venous tone | Blood flow velocity, cm/sec |
|---|---|---|---|---|---|
| 1 | 0.28 | 65 | 16 | 0.15 | 2.72 |
| 2 | 0.25 | 55 | 18 | 0.16 | 2.72 |
| 3 | 0.2 | 73 | 14 | 0.1 | 2.79 |
| 4 | 0.22 | 60 | 13 | 0.14 | 3.06 |
| 5 | 0.16 | 61 | 17 | 0.1 | 2.72 |
| 6 | 0.2 | 68 | 12 | - | 3.3 |

After flight

| Time of examination, day | Arterial tone | Arterial pressure, mm Hg | Venous pressure, mm Hg | Venous tone | Blood flow velocity, cm/sec |
|---|---|---|---|---|---|
| 1st (morning) | 0.25 | 60 | 12.5 | 0.057 | 4.48 |
| 1st (evening) | 0.16 | 70 | 18 | 0.07 | 3.06 |
| 2nd | 0.14 | 70 | 17 | 0.05 | 3.5 |
| 4th | 0.1 | 75 | 21 | 0.032 | 6.4 |
| 5th | 0.14 | 67 | 20 | 0.035 | 4.42 |

As regards the problem of the possible causes of dilatation of the skin vessels, it is pointed out that an increase in the cross-section of the arterial and venous vessels, as well as an increase in the rate of blood flow should, strictly speaking, be associated with an increase in the air temperature by 2-4°C in the experimental chamber.

## State of the Peripheral Blood Circulation of the Dog Malek During a Rocket Flight

Examination of the animal before the flight showed that the arterial pressure varied between limits which are characteristic for healthy animals - 75-80 mm Hg (Table 2). During the initial period of the flight, when the overloading factor was operating, the maximum arterial pressure increased to 107 mm Hg.

During the period when weightlessness sets in it still remained high (111 mm Hg).

Table 2

| No. of examinations | Arterial pressure, mm Hg | Venous pressure, mm Hg | Arterial tone | Venous tone | Blood flow velocity, cm/sec | Remarks |
|---|---|---|---|---|---|---|
| Before flight | | | | | | |
| 1 | 75 | 40 | 1.0 | 0.09 | 0.9 | |
| 2 | 80 | 35 | 1.0 | 0.09 | 1.2 | |
| 3 | - | 30 | 1.0 | 0.08 | 2.48 | |
| 4 | 80 | 20 | 1.0 | 0.095 | 1.08 | |
| During flight | | | | | | |
| | 107(35) | 26(10)<br>40(70) | 1.0(0-20)<br>0.5(40-70) | 0.09(20)<br>0.095(80) | 0.88(10)<br>1.44(70) | Overload period |
| | 111(155) | 16(130) | 1.0(90-220) | 0.08(140) | 0.65(130) | Period of weightlessness |

Note: The numbers in parentheses designate the second at which the measurements were made after launching.

When examining dogs under ground conditions, the venous pressure was relatively high at the beginning of the experiment (40 mm Hg), then became normal and finally decreased to 20 mm Hg. During the flight, particularly during the period of acceleration, an increase in the pressure from 26 mm Hg in the tenth second of the flight to 40 mm Hg in the 70th second occurred. The venous pressure decreased to 16 mm Hg during the period when the animal was in a state of weightlessness.

It can be seen from Table 2 that prior to the flight the arterial tone was high during the entire investigation. A high arterial tone was also noted during the first 20 seconds of the flight but from the 40th second onward, it decreased sharply. This lowering of arterial tone was recorded up to the 70th second, when it then started to increase and remained high until the end of the observation period (180 seconds).

The blood flow velocity, as well as the venous tone, did not change appreciably during the flight.

On the basis of this data, the three indices denoting the state of the peripheral vessels which changed during the animal's flight in the rocket, were: the maximum arterial pressure, the venous pressure and the arterial tone.

From the time when the rocket left the launching pad the air temperature increased in the section of the rocket where the animals were housed. It is this factor in particular which can explain the dilation of the skin blood vessels and the drop in the arterial tone which occurred from the 40th to the 70th second of flight. However, during the entire subsequent period when the air temperature in the capsule continued to increase (i.e. the 71st second of flight onward) a spasm of the blood vessels was recorded which lasted until the end of the period of plethysmograph tracing. Therefore, the dilatation of the vessels and their decrease in tone do not appear to be the result of the increased air temperature in the capsule but the result of acceleration during the flight.

The high increase in the venous pressure during the period of overloading can apparently be explained by the purely mechanical sluggishness of the blood in the vessels under the influence of acceleration in the distal direction with respect to the ear cavity of the animal.

When summarizing the data from the investigations on the reactions of the peripheral vascular system of the dogs Malek and Belka during and after flight into space, it is important to point out that neither in the first nor in the second case were any appreciable disturbances observed in these peripheral vessels. A number of authors (Galkin, Gorlov et al., 1958; Henry, Ballinger et al., 1952) have shown that arterial and venous pressures and the pulse rate change to a much lesser extent if the animals fly in a state of narcosis. This may indicate the importance of the role of the cortex in the observed hemodynamic changes.

SUMMARY

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Maximum arterial pressure, venous pressure, arterial and venous tone, and the blood stream rate in the peripheral blood vessels (of ears) of dogs have been measured by a universal manometer designed by the author. Data obtained during space flights in rockets and satellites indicate that venous pressure increased during overloads, but during the weightless period both venous and arterial pressure were normal. Shifts in arterial and venous tone and the blood stream rate were not significant.

Author

[Translator's note: This is an original abstract and not a copy of the English "summary" printed with the article.]

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# OBSERVATIONS ON THE CONDITIONED REFLEX ACTIVITY OF WHITE RATS SOME CONSIDERABLE TIME AFTER TRAVELLING ON THE SECOND SOVIET SATELLITE

L. D. Luk'yanova

Investigations of Soviet and other scientists during rocket flights into the upper layers of the atmosphere and study of the combined effects of various factors during and after flight in Soviet spaceships on living organisms made it possible to study in detail many physiological functions in these conditions.

An important aspect of research into space physiology is the study of the effects of the conditions of space flights on the function of the central nervous system, in particular, on higher nervous activity (HNA).

Study of the HNA of animals in conditions of sharply changed environment has become a tradition of Russian physiology. During recent years, foreign scientists have also paid attention to this problem (Hetherington, 1959).

One of the objects in investigating the effect of space flight on primates carried out in the USA on the rocket "Jupiter C" was to study the effect of flight on the motor conditioned defence reflex. However, this part of the investigation was not carried out during the flight. As far as can be judged from the published material (Graybiel, McWinch and others, 1960; Gerathewohl, 1960; Nixon and others, 1960), the HNA was also not investigated after the flight on the only monkey, Baker, who lived for a long time after the flight.

The object of our work was to study the effect of flight in a spacecraft on the HNA.

The experiments were carried out on white male rats by the motor-food technique of Kotlyarevskiy. Preparation of the animals was begun six months before the flight. A stereotype of six positive conditioned reflexes from the auditory and visual analyzers and one differentiating reflex were formed and reinforced in the animals. Tests were carried out on the type of HNA of the animals. The results of 30 experiments, carried out after consolidation of the stereotype, were used to define the initial conditioned reflex background. The results obtained for each animal were processed statistically.

At the same time observations were made on the morphological composition of the peripheral blood, the weight and general condition of the animals.

As already reported in the press, the second Soviet satellite carried two rats. They were placed in a special cage[1]. The control group consisted of 5 rats which were brought to the launching pad together with the experimental rats and were kept there during the flight.

The experiments on the test and control rats were begun 4 days after landing and continued for 8 months.

The rats endured the flight well: they kept their original weight and were normally mobile. A considerable proportion of the food placed in the feeding trough was consumed. Examination of the peripheral blood showed no significant changes as compared with the initial level and the control group. A certain increase in the absolute number of lymphocytes in the test rats was within the limits of individual variation of this factor in the control rats.

Soon after return to Earth, test rats Nos. 12 and 18 showed certain changes in HNA.

Rat No. 12 with a strong, excitable type of HNA, showed during the first three days shortening of the latent period in response to a strong sound stimulus and a longer latent period in response to a weak light stimulus, with impairment of differentiation. The length of the latent periods returned to normal within 20 days. The intensity of the conditioned reflexes to positive stimuli remained within normal limits (Figure 1).

Successive inhibition was observed 20 days after landing in an experiment with starvation for two days, whereas positive induction was observed in the same conditions before the flight.

However, other functional tests, namely the caffeine test, external inhibition, extinction and restoration, showed no changes in the functional state of the cortex and the isolated effect of an inhibitory stimulus was tolerated even better. Thus before the flight, in this rat, differentiation was disinhibited after exposure for 12 seconds to an inhibitory stimulus, while after the flight in the spacecraft the reaction was observed after 70 seconds.

The second experimental rat (No. 18) with a strongly equilibrated type of HNA, from the very first day after return showed in the laboratory a distinct rise in positive conditioned reflexes and shortening of their latent periods. However, unlike the previous rat, this one displayed a deepening of differentiation. The failure percentage of

[1]See page 450 of the article "Ensuring living conditions for mice during prolonged flight in spaceships and satellites", pp. 449-452 of this volume.

conditioned reflexes was reduced. Return to normal was observed in 20 days (Figure 2).

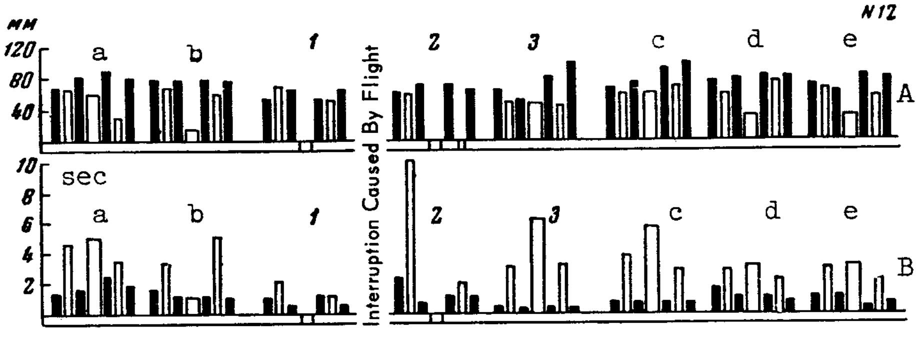



Figure 1. Change in the Conditioned Reflex Response Before and After Flight (Rat No. 12). A - intensity of conditioned reflexes, mm; B - latent period of conditioned reflexes, sec; a, b, c, d, e - mean values of conditioned reflexes for 10 experiments; 1 - last individual experiment before interruption caused by the flight; 2, 3 - first and second experiments after return. Reflexes arranged in stereotype order. Black columns - reflex to sound stimulus; narrow white columns - reflex to light stimulus; wide white columns - reflex to differentiating stimulus; columns directed downwards - no reflex to stimulus.

The experiments showed that starvation for 2 days and the caffeine test gave results somewhat different from those before the flight. Before the flight the reflexes remained normal in these experiments, differentiation was not disinhibited, and the number of inter-signal reactions slightly increased, whereas after the flight, 2-day starvation and the caffeine test sharply increased all the reflex responses, the number of inter-signal reactions and also disinhibited differentiation. This was apparently not related to weakening of the inhibitory process but to an increase in the excitation process, since in other respects the concentration of the inhibitory process was increased (positive induction, weakly marked before the flight, was conspicuous after it).

The other functional tests, namely lengthening of differentiation, external inhibition, extinction and restoration, gave the same results as before the flight in the spaceship.

To decide whether the changes observed in the HNA of the rats were the result of the flight in the spacecraft or whether they were due to associated factors (transportation, climatic conditions, interruption in

the work, etc.) it was necessary to compare the dynamics of the HNA of the test rats with those of the control animals.

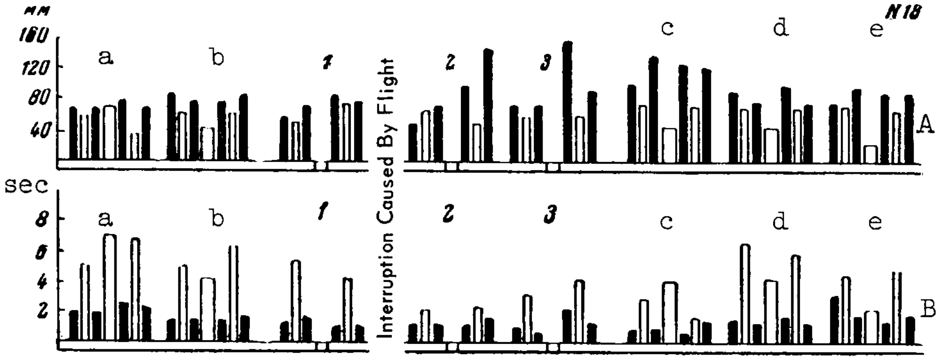



Figure 2. Change in Conditioned Reflex Activity Before and After the Flight (Rat No. 18). A - intensity of conditioned reflexes, mm; B - latent period of conditioned reflexes, sec; a, b, c, d, e - mean values of conditioned reflexes for 10 experiments; 1 - last individual experiment before interruption caused by the flight; 2, 3 - first and second experiments after return. Reflexes arranged in stereotype order. Black columns - reflex to sound stimulus; narrow white columns - reflex to light stimulus; wide white columns - reflex to differentiating stimulus; columns directed downwards - no reflex to stimulus.

Analysis of the conditioned reflex activity of the control rats after resumption of work with these animals enables us to divide them into two groups.

To the first group two rats were assigned (Nos. 11 and 19 - strong non-equilibrated and equilibrated types of HNA) in which the changes in HNA after the return were slight. The rats of this group showed shorter latent periods of positive conditioned reflexes, and rat No. 11 increased its intensity of positive reflexes (Figure 3). Differentiation in both rats in the first one or two days after return was disinhibited but then restored. By the end of the third month the number of experiments in which phase states were noted had risen with increase in the percentage failure of positive conditioned reactions. The tests with 2-day starvation gave the same results as in normal conditions. However, administration of caffeine was found to reduce the conditioned reflex activity. The number of inter-signal reactions sharply fell below the initial level (after a month, by 2.5 times), but at the end of the third month it had again slightly increased. Rat No. 11 showed somewhat better

tolerance of the isolated effect of an inhibitory stimulus after the return.

The second group of control animals included rats Nos. 14 (weak inhibited type of nervous system), 15 (strong non-equilibrated type) and 17 (strong equilibrated).

A characteristic feature of this group was the more or less appreciable fall in conditioned reflexes. Rats Nos. 14 and 17 in the first few days after interruption were found to have reduced positive conditioned reflexes with longer latent periods (Figure 4). The number of instances of phase states was increased with more failures of conditioned reflexes and disinhibition of differentiation. The results of the caffeine test were adversly affected, as were those of the test carried out with rat No. 14 starved for 2 days.

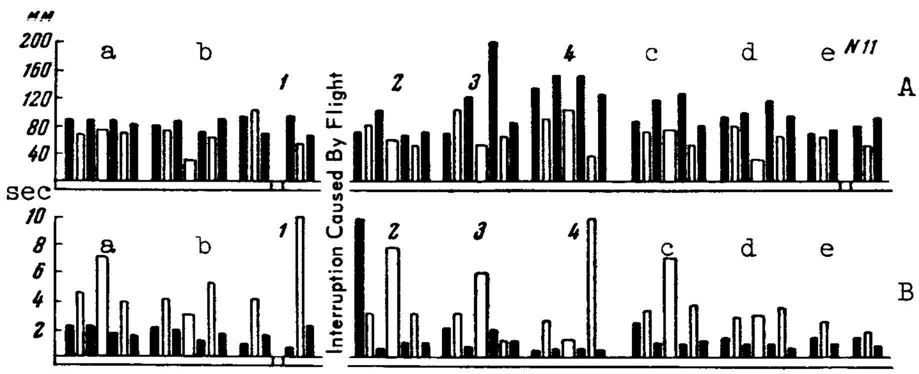



Figure 3. Change in Conditioned Reflex Activity Before and After the Flight (Rat No. 11). A - intensity of conditioned reflexes, mm; B - latent period of conditioned reflexes, sec; a, b, c, d, e - mean values of conditioned reflexes for 10 experiments; 1 - last individual experiment before interruption caused by the flight; 2, 3 - first and second experiments after return. Reflexes arranged in stereotype order. Black columns - reflex to sound stimulus; narrow white columns - reflex to light stimulus; wide white columns - reflex to differentiating stimulus; columns directed downwards - no reflex to stimulus. 4 - 3rd experiment after return.

Cortical activity returned to normal after 2 months only in rat No. 17. In the rats of this group the response to lengthening the time of the isolated action of an inhibitory stimulus was not changed. The number of inter-signal reactions was slightly reduced.

During the first few days after return rat No. 15 showed a marked reduction in motor activity, and a considerable number of reflexes were inhibited. However, during the subsequent period the state of the HNA of this rat improved with restoration to normal of conditioned reflexes to a powerful stimulus, although the conditioned reflex to a weak stimulus remained low with fairly frequent failures. The results of the test with 2-day starvation did not differ from those under normal conditions. The number of inter-signal reactions decreased.

Thus, comparison of the conditioned reflex activity of the test and control animals shows that during the first two months the changes in the HNA of rats carried in the spacecraft were not greater than in the control group. The test rats showed no manifestations absent in the control animals.

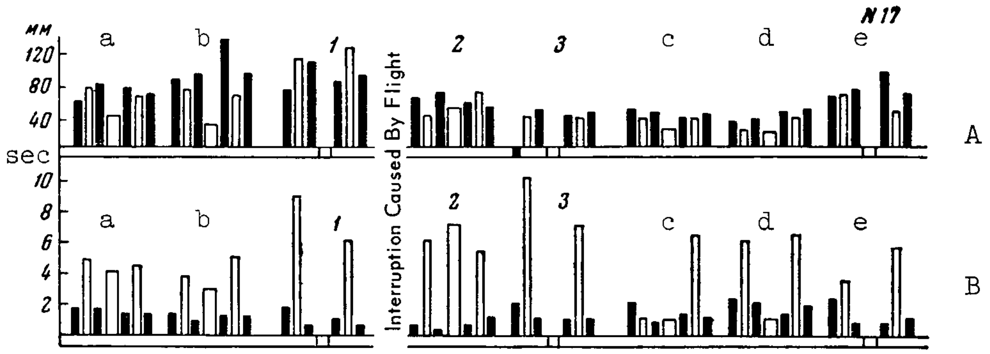


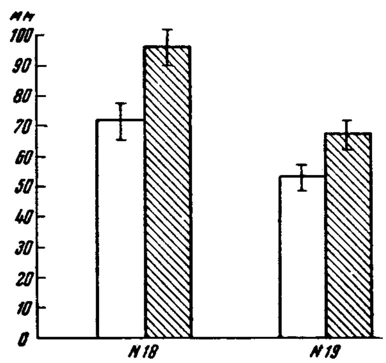

Figure 4. Change in the Conditioned Reflex Activity Before and After the Experiment (Rat No. 17). A - intensity of conditioned reflexes, mm; B - latent period of conditioned reflexes, sec; a, b, c, d, e - mean values of conditioned reflexes for 10 experiments; 1 - last individual experiment before interruption caused by the flight; 2, 3 - first and second experiments after return. Reflexes arranged in stereotype order. Black columns - reflex to sound stimulus; narrow white columns - reflex to light stimulus; wide white columns - reflex to differentiating stimulus; columns directed downwards - no reflex to stimulus.

As stated, the test rat No. 18 showed a statistically reliable increase in the reflex to a strong stimulus. However, a similar picture was observed in control rat No. 19 (Figure 5).

Disinhibition of differentiation noted in the first few days after return in test rat No. 12 was also observed in four of the five control rats.

Lengthening of the latent period to some stimuli, found for the test rat No. 12 in the first three days, was recorded in the control rats Nos. 14 and 17.

The results of the 2-day starvation test were less unfavorable in test rat No. 12 than in control rat No. 14. The initial conditioned reflex background was restored in some of the control rats in 2 months after return to the laboratory, i.e. even later than in the rats of the test group. It should be noted that certain difficulties associated with the main experiment (unavoidable interruption in the work, the effect of meteorological factors, transport, etc.) Must have had some effect on the animals. This was vividly seen in the control rats (Nos. 14 and 17) of the second group. In these animals the basic nervous processes seemed to have been weakened so that the previously well tolerated stereotype of conditioned stimuli was on return a source of extreme stress, causing changes in the activity of the higher sections of the central nervous system.




(experimental) (control)

Figure 5. Change in the Value of the Reflex in Rats to a Sound Stimulus Before and After the Flight. White column - before the flight or journey to the launching pad; shaded column - after return from the launching pad. Vertical lines in the columns denote 3 standard deviations.

After restoration of the initial conditioned reflex background the higher nervous activity remained normal for 6 months and the variations in conditioned reflexes to positive stimuli and reactions to differentiation did not exceed the variations in these factors under normal conditions in healthy animals (Figures 6, 7).

In the eighth month after the flight the test and control rats began to show outward signs of aging (loss of hair, decline in motor activity and weakened food excitability). This was accompanied by a distinct deterioration in HNA: fall in positive conditioned reflexes,

longer latent periods, increased percentage in failure of reactions and phase states.

These manifestations were much the same in the test and control rats and developed at an early stage. At the end of the eighth month one control rat (No. 14) and one test rat (No. 18) died from natural causes.

The small number of animals limits the possibility of evaluating the results of the experiments and drawing final conclusions. Verification of the results on more extensive material with use of more sensitive criteria of the working efficiency of the higher sections of the brain, is a future task which will entail a great deal of work. However, even now we may conclude that flight in the spacecraft did not produce marked disturbances in the HNA of our test animals (during the observation period).

The vast experience gained by Soviet physiologists in study of the effect of various factors on the higher sections of the central nervous system by the conditioned reflex method shows that if any of these factors produces severe disturbances in HNA, they are seen in only a small number of animals.

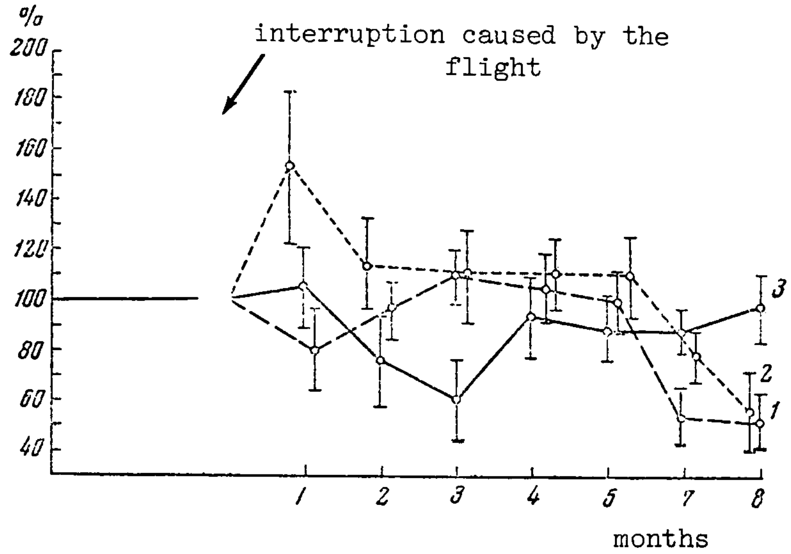



Figure 6. Reflex to Sound Stimulus in Rats (Values Obtained After the Flight are Expressed as Percent of the Initial Level). 1 - rat No. 12. 2 - rat No. 18. 3 - rat No. 14. The period after the flight was 8 months. The vertical lines are multiplied by 3. Abscissa - time, months; ordinate - intensity of reflex, percent.

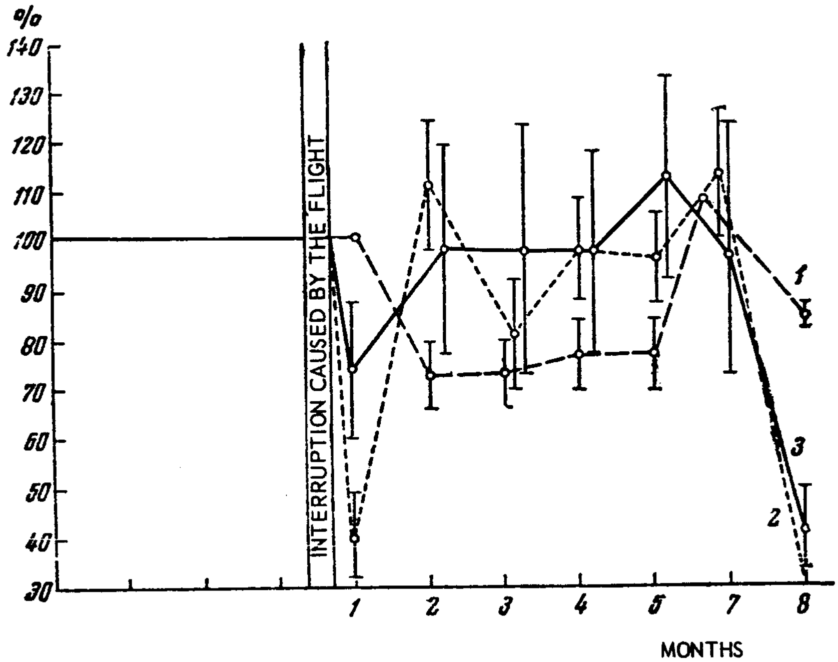



Figure 7. Length of Latent Period to a Weak Stimulus (Light) in Rats Before and After the Flight. Ordinate - length of latent period, percent. Notes as in Figure 6.

It is not by chance that most of the work done on the effect of removing various sections of the nervous system or certain glands of internal secretion on conditioned reflexes has been carried out on two to five dogs.

A number of studies show that food conditioned reflexes are an extremely sensitive indicator of the effect of unusual environmental conditions. Thus, M.P. Brestkin (1960) et al. showed that on exposure to accelerations and hypoxia, the food conditioned reflexes are much more severely disturbed than the defense acid ones. Similar conclusions were reached by P.I. Lomonos (1956) who studied the effect of ionizing radiation on the HNA of dogs. After exposure to radiation producing a sharp fall in the food conditioned reflexes, the acid reflexes were maintained and frequently enhanced. The same phenomenon was seen by D.Ya. Glezer (1948) when dogs were exposed to intensive ultra-high frequency fields.

The absence of a significant difference between the food conditioned reflexes in rats carried in the second Soviet satellite and in the control rats exposed merely to factors incidental to space flight suggests that the flight in the second satellite had no markedly harmful effect on the functioning of the higher sections of the brain.

In conclusion, acknowledgments are made to N. N. Livshchits, who suggested and supervised this investigation.

Summary

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The combined effect of various space-flight factors on the higher nervous activity was investigated on seven male albino rats, of which two participated in the flight and five served as controls. A stereotype of six positive and one differentiated motor-alimentary conditioned reflexes from optical and acoustic analyzers was produced in all the animals; the composition of the peripheral blood, the weight and the general state were determined and the data were used for statistical processing. For two and a half months after the flight no changes could be detected in the higher nervous activity of the rats studied, they were only slightly different from those of the controls. It is concluded that the animals withstood satisfactorily the conditions of space flight. Author

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# INFLUENCE OF SPACE-FLIGHT FACTORS ON THE BACTERICIDAL ACTIVITY OF THE BODY

O. G. Alekseyeva and A. P. Volkova

During space flight and during the launching and landing of rockets, the body of the astronaut is subjected to the influences of a number of physical factors. It is known that one of these (ionizing radiation) has, within certain doses, an important influence on the state of the natural resistance to bacterial infection. By simulating the effects of these physical factors experimentally, Soviet and non-Soviet investigators have established that many of these cause a change in the functional state of the nervous and the cardiovascular systems. Due to this it is likely that they can cause a change in the degree of natural resistance, particularly since the microflora of the pressurized capsule of the rocket may differ from the microflora of the terrestrial environment.

In studying the reaction of the organism to the many physical factors which accompany space flights, methods were used from which a sufficiently complete understanding of the state of the natural resistance with respect to a challenge with pathogenic bacteria could be obtained.

Since small dogs weighing 4-6 kg are used in space flights, the normal bactericidal competence of 32 dogs of similar weight was examined without applying any of the test stresses. To obtain at least an approximate idea of the character and intensity of the influences on immunity of the basic physical factors that accompany launching and landing of a rocket, laboratory experiments were made with dogs in a centrifuge and in a vibration test-stand. Furthermore, dogs that had flown in rockets, which had taken off vertically during the past year, were kept under observation for a year.

The main experiment consisted of making observations on six dogs which had flown in 1960-1961 in either a geophysical (vertical take-off) rocket or in satellites. The dogs were inspected directly before the launching and after landing of the rocket by T. S. L'vova, to whom acknowledgments are made.

## Methods

Experiments were made on the influence of 70 cps vibration of 0.4 mm amplitude for a duration of 13 min. The influence of acceleration was investigated in a centrifuge. The acceleration was increased to 2, 6, 9 and 12 g for 1-2 min.

The microflora of the skin was studied by taking imprints on plates (with an area of 6 $cm^2$) containing a modified Korostelev medium. It is pointed out that in contrast to the composition of the medium described in our earlier works[1], the concentration of bromthymol blue was raised to 5 percent. This was done in order to suppress the growth of all bacteria strains except staphylococci which are able to grow at this concentration of bromthymol. Therefore, we have only taken into consideration the number of staphylococci on the skin and not the total number of bacteria because there are so many on a dog's skin that they would fuse into a solid mass ("lawn") on the plate. The microflora of the oral cavity was investigated according to the method of G. A. Shal'nova. This method consists of removing bacteria from the mucous root of the tongue by means of a sterile standard disc of filter paper. The disc was then ground in a mortar with 5 ml of a physiological solution and 0.1 ml of the suspension was poured onto a Petri dish containing Endo medium and blood agar. After incubation, the number of colonies on the plates was counted and the type, composition of the flora and the percentage of haemolytic strains were determined.

The bactericidal properties of the skin were determined according to the method of N. N. Klemparskaya, which has already been described.

We used freshly secreted strains of the intestinal Bacillus No. 1 as test bacteria. These were grown daily in broth culture from a 1:5000 dilution of cells. The rate at which the cells died off was investigated 10 and 20 min after dubbing the skin. The number of colonies imprinted on the entire plate of 6 $cm^2$ area was counted.

The phagocytic and bactericidal properties of the blood were determined according to the method of V. M. Berman and Ye. M. Slavskaya. The intestinal Bacillus No. 675, which had been obtained from the L. A. Tarasevich Control Institute was used as the test strain. The method for reducing the blood to the required volume was somewhat modified. The initial mixture consisted of 0.03 ml of 5 percent sterile citrate, 0.1 ml of blood and 0.03 ml of suspension of the daily culture of test bacteria.

The suspension in isotonic solution contained $2.5 \times 10^2$ cells per 1 ml. The first incubation lasted for 30 minutes and the second, on agar, for 2 hours. The extent (during both phases) of phagocytosis was determined from the average number of phagocytosed or digested bacilli per counted

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[1]N. N. Klemparskaya and O. G. Alekseyeva. Meditsinskaya radiologiya, No. 3, 1959, p. 70.

number of neutrophils. This enabled the phagocytic number (the average number of phagocytosed bacilli per one active neutrophil) and the percentage of active, i.e. phagocytic neutrophils to be calculated. The bacterecidal properties of the plasma were estimated from the percentage of bacilli digested extracellularly.

## Results

Experiments made after the animals had been subjected once to the stresses of acceleration and vibration under laboratory conditions have shown that these effects do not have an appreciable influence on the microflora of the skin but in the same way as in "stress" they bring about certain changes in its bactericidal activity. The most sensitive of the tests investigated proved to be the phagocytic action of the neutrophils of the blood. Ten to fifteen minutes after the end of exposure to vibration, a decrease was observed in the absorptive properties of the neutrophils in three of the six dogs. In the other three, an increase in the ability to absorb was accompanied by a fall in the ability to digest.

For days after the vibration test, eight dogs were examined and in all of them a decrease in the capacity of neutrophils to absorb cells was observed. In dogs which had never been under space-flight conditions, the decrease in the ability to complete the first phase of phagocytosis was accompanied by a depression of the digestive ability of the blood neutrophils. In three out of four dogs that flew in geophysical rockets previously, the decrease in absorption was compensated by activation of the digestive action of the phagocytes.

A dependence on the magnitude of acceleration was observed. Thus, in the case of an overload of 2 g, activation of both phases of phagocytosis was observed in the dog Lisenka. Before the experiment 0.44 bacteria were phagocytosed per calculated neutrophil. Twenty percent of the bacteria cells had been digested. During the period of centrifugation the phagocytic number increased to 0.72 and the refraction digested to 25 percent. At the end of the test, these values remained at the high levels of 0.7 and 37 percent.

During the application of overloads of the order of 6 g, different reactions of the phagocytic function of the blood neutrophils were observed in the individual dogs. In four of the experimental animals the absorption function was compensated for by an activation of the digestive function of the neutrophils. Thus, for instance, in the dog Otvazhnaya there was an average of 0.3 phagocytosed bacteria, of which 20 percent were digested for each neutrophil. After overloads of the order of 6 g, there were only 0.22 bacteria per neutrophil but 45 percent of the bacteria were digested, which not only compensated for the decrease in the absorption bacteria but even led to an increase in the absolute number of

bacteria digested in the phagocytes. In all other animals (also four dogs) a depression of both phases of phagocytosis was observed.

When an acceleration of 9 g was applied, a decrease was observed in the phagocytic reactions of the four experimental dogs. This occurred in two of the dogs during acceleration in the centrifuge, and in the other two after the centrifuge was stopped.

At overloads of the order of 12 g there was a depression of phagocytosis in two dogs, in one dog the phagocytic reaction did not change appreciably and only in the dog Tsygan was an activation of the absorption function of the neutrophils observed. However, even in this dog the digestive ability of the phagocytes was not reduced.

The bactericidal properties of the blood were more stable to overloads and depression of activity could be observed only at accelerations up to 12 g. After vibration and acceleration, the change in the bactericidal properties of the skin did not follow a regular pattern.

The occurrence of a seasonal decrease in the number of bacteria on the skin which are resistant to bromthymol blue, was observed previously in the dogs Belka and Strelka after their flight in the second Soviet satellite. The bactericidal activity of the skin against intestinal bacilli was very pronounced during the first weeks. This then reverted to the usual intensity for the given season. In the same way as in the laboratory experiments, the most sensitive of all the tests proved to be the phagocytic reaction. A moderate activation of the phagocytosis of the intestinal bacilli was observed for the dog Strelka from the ninth day – and in the dog Belka from the eighteenth day after landing. The activation was prolonged and had not ceased even a month after the flight. In the dog Strelka it was accompanied by an absolute activation of the digestive function of the phagocytes (Table 1). During the first two weeks after the flight, a tendency for the bactericidal properties of the plasma to be activated was also observed.

After a short duration space flight on the satellite, the dogs Chernushka and Zvezdochka also showed changes in their bactericidal activity. These changes were less pronounced than in the dogs Belka and Strelka who accomplished a longer flight in space. Thus, activation of the bactericidal properties of the skin of the dogs Chernushka and Zvezdochka occurred only during the second, fourth and seventh day after the flight. The primary drop in phagocytosis was more pronounced and activation of the phagocytic reaction of neutrophils and the bactericidal properties of the blood was less intense. A similar blood reaction was also observed in the control dog Kometa. This dog was a standby astronaut, which went through the same training as the other dogs and although brought to the launching pad did not fly. Therefore, it cannot be ruled out that the changes in the bactericidal and phagocytic properties of the

blood of Chernushka and Zvezdochka were due not so much to the flight effects as to a change in the climatic and other environmental conditions. However, no activation of the bactericidal properties of the skin was observed in the control dog (Figure 1). Consequently, no changes observed in the dogs Chernushka and Zvezdochka were clearly associated with the flight. An additional bacterial test was made on this batch of dogs, in which the microflora of the oral cavity was examined. It has already been mentioned that the microflora of the skin were relatively resistant to the "stress" effects that accompany space flights.

Table 1. Change in the Phagocytic Reactions of the Dogs Belka and Strelka After Their Flight in the Second Soviet Satellite

| Average No. of bacteria per counted neutrophil | April 1960 | June 1960 | Belka | | | | Strelka | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Days after flight | | | | Days after flight | | |
| | | | 9 | 18 | 24 | 31 | 9 | 18 | 31 |
| Phagocytic (Phase I phagocytosis) | 0.34 | 0.44 | 0.12 | 0.88 | 0.7 | 0.56 | 0.92 | 0.76 | 0.48 |
| Digested (Phase II phagocytosis) | 0.14 | 0.22 | 0 | 0.26 | 0.12 | 0.12 | 0.56 | 0.42 | 0.06 |
| Bacteria digested in the plasma (bactericidal properties of the plasma), % | 24 | 24 | 24 | 40 | 20 | 8 | 40 | 29 | 8 |

Note: In dogs of this weight not subjected to overloading, the average number of phagocytosed bacteria varied between 0.2 to 1.0 (arithmetic mean 0.61) and the number of digested bacilli - from 0 to 0.5 (arithmetic mean 0.16).

It can be assumed that the flora of the oral cavity, which are more intimately associated with the body of the animal, will change soon after the flight. The bactericidal activity of these animals changed, and it is not difficult to see that these changes would probably influence the state of the "autoflora".

The assumptions of the authors proved justified. In both astronaut dogs the intestinal bacillus (Escherichia coli) appeared in large numbers in the oral cavity after the first two days of flight, a phenomenon which had not been observed either before flight or subsequently. It was not observed in other dogs. In some of the control dogs after their return

from the launching pad, as well as in the dogs Chernushka and Zvezdochka, there was an increase in the total number of cocci in the oral cavity. Intestinal bacteria, however, were not found.

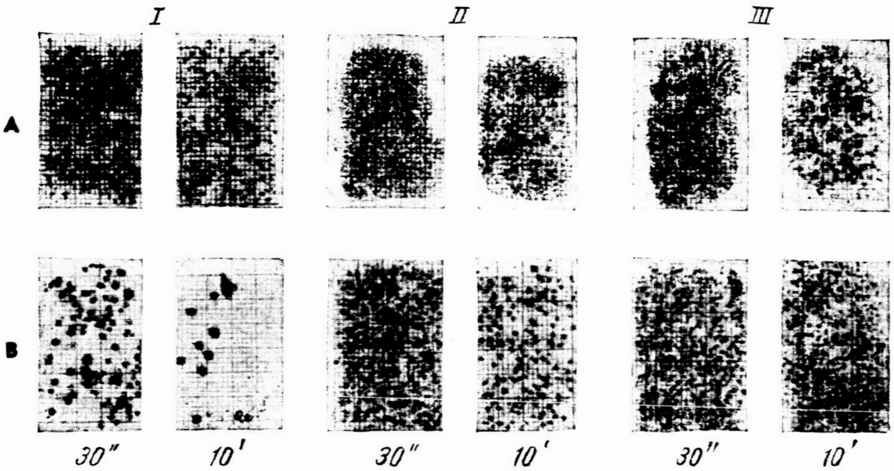


Figure 1. Photograph of Imprints Used in Determining the Bactericidal Properties of the Skin of the Dogs Chernushka (I), Zvezdochka (II) and Kometa (III)
A - before the flight; B - second day after the flight. At the bottom is given the time of taking the imprints after dubbing the skin with the culture of the intestinal bacillus is given. The smaller the number of colonies (black dots), the more bacteria died on the skin and the more intensive was the bactericidal property of the skin.

In the dogs Malek and Otvazhnaya a wavelike change in the bactericidal properties of the skin was observed after their flight in the geophysical rocket which was launched in a vertical trajectory. During the first two weeks after the flight a decrease was observed in the number of bacteria which were normally stable to bromthymol blue. During the first hours after the flight, a decrease in bactericidal activity was observed which after the third week was reversed through the activation of the phagocytic and bactericidal properties of the blood (Table 2). It is pointed out that all these phenomena were more pronounced in the dog Malek than in the dog Otvazhnaya. In the view of the authors this was due to the fact that the dog Otvazhnaya had made several flights in recent years. During laboratory examination of the dogs it was found that those which had previously flown in vertical launched rockets reacted to a lesser extent to "stresses" than the dogs which had not flown. In some degree this indicated a higher level of training in these animals. However, in the quiet state, without applying any loads, their bactericidal activity was at the lower boundary of the physiological norm. It is

possible that the decreased reaction to new "stresses" in animals who had already flown into space was associated with the stable decrease in their bactericidal activity.

Table 2. Changes in the Phagocytic Reactions of the Dogs Otvazhnaya and Malek after They Had Flown in a Geophysical Rocket

| Average No. of bacteria per counted neutrophil | Otvazhnaya | | | | | | |
|---|---|---|---|---|---|---|---|
| | Before flight | | | After the flight | | | |
| | Feb. | March | Eve | 2 hrs | 14 days | 30 days | 70 days |
| Phagocytosed (Phase I phagocytosis) | 0.34 | 0.3 | 0.12 | 0.14 | 0.04 | 1.14 | 0.44 |
| Digested (Phase II phagocytosis) | 0.14 | 0.06 | 0 | 0.02 | 0 | 0.4 | 0 |
| Bacteria digested in the plasma (bactericidal properties of the plasma), % | 30 | 34 | 20 | 40 | 24 | 32 | 26 |

| Average No. of bacteria per counted neutrophil | Malek | | | | | | |
|---|---|---|---|---|---|---|---|
| | Before flight | | | After the flight | | | |
| | Feb. | March | Eve | 2 hrs | 14 days | 30 days | 70 days |
| Phagocytosed (Phase I phagocytosis) | 0.26 | 0.18 | 2.24 | 0.28 | 0.66 | 1.74 | 1.52 |
| Digested (Phase II phagocytosis) | 0.04 | 0.04 | 0.32 | 0.14 | 0.22 | 0.54 | 0.76 |
| Bacteria digested in the plasma (bactericidal properties of the plasma), % | 16 | 12 | 18 | 32 | 42 | 30 | 56 |

Thus, space flights bring about wavelike changes of moderate intensity in the bactericidal responses of dogs. To illustrate the point, the variation response curves are reproduced which were obtained when studying phase I phagocytosis (Figure 2). It can be seen from the figure that firstly all fluctuations in the phagocytic index after flights did not exceed the values possible for "terrestrial" variants. Secondly, three

phases can be observed: during the first week after the flight, the phagocytic index is low; from the second to the seventh week a moderate activity in the phagocytic function of the neutrophils can be observed; lastly, some considerable time after the flight the phagocytic index again decreased to the lower "norm" level.

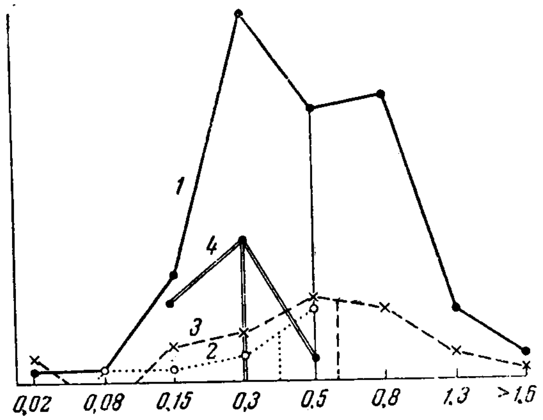


Figure 2. Variation Response Curves Obtained when Studying the Phagocytic Function of Blood Neutrophils at Various Times after the Flight
Along the ordinate - number of observations in each group; along the abscissa - phagocytic index. 1 - "intact" dogs; 2 - after one week; 3 - after 2-7 weeks; 4 - from 7 weeks to 3 years. The perpendicular lines indicate the mean arithmetic values of the phagocytic index for each group of dogs.

The data obtained from other tests differed somewhat from the character of the changes observed in the phagocytic index but they also revealed wavelike changes. Thus, for instance, the bactericidal properties of the skin, during the first week after the flight increased and then tended to fall off again, were observed. However, some considerable time after the flight there may again occur an increase in the bactericidal properties of the skin. Thus, no low level of this function was observed in any of the twelve investigations of the bactericidal properties of the skin of dogs which had flown in geophysical rockets during past years (at least 65 percent of the bacteria perished in 10 minutes). In 10 animals out of the 32 which had never flown the bactericidal property during this season of the year was relatively low.

Taking into consideration that after the flights the animals on the whole remained practically healthy and that the intensity of the

bactericidal changes was moderate, the authors are inclined to think that this represents an adaptation reaction to the effects of new physical factors. There may even be a moderate overadaptation but no pathological change occurred in the natural immunity of these dogs after the space flights.

## Conclusions

1. The bactericidal activity of dogs can change after being subjected to a single excessive treatment with vibration (in a centrifuge).

2. After space flights on satellites and geophysical rockets, a moderate wavelike change in the bactericidal activity occurred which during the first weeks was accompanied by a disturbance of the state of the autoflora of the skin and oral cavity.

3. The changes in the bactericidal activity appear to be an adaptation reaction to the effects of the many physical factors that accompany space flight.

## Summary

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Dogs exposed to space-flight factors exhibit a wavelike fluctuation of the phagocytic index. The ingestive ability of neutrophils was most affected. The digestive ability of neutrophils and the bactericidal function of plasma and skin were less affected. The appearance of Escherichia coli in the oral cavity in the immediate postflight period indicates a drop in the immunological activity. Similar changes in the immunological indices were obtained in ground tests after vibrations (70 cps, 0.4 mm amplitude) for 13 minutes and centrifuge accelerations (2, 6, 9 and 12 G) for one to two minutes. Dogs which have been exposed to space flight on previous occasions were less sensitive and showed less pronounced immunological changes, indicating an adaptation to the combined effects of space-flight factors. Moderate immunological changes persisted in all dogs for months and sometimes years.

Author

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# EFFECT OF SPACE-FLIGHT CONDITIONS IN THE SATELLITE ON THE PRESERVATION OF THE VIABILITY OF CHLORELLA CULTURES

V. Ye. Semenenko and M. G. Vladimirova

In the conquest of space, crews may fly for many months and possibly years and may land on other planets. As one of the methods of insuring life support, it is proposed to create inside the cabins an ecological system which will simulate, to a certain extent, the conditions of life on Earth (second Soviet spacecraft, 1960; Gyurdzhian, 1961; Mangelsdorf, 1959; Stile, 1960; Nakamura, 1959).

The photosynthetic abilities of green plants will play an important role, particularly the photosynthesis of microscopic single-cell algae, which can be utilized for regenerating the air as well as for supplying food to the crew (Nakamura, 1959; Nichiporovich, 1961; Strughold et al., 1959). Therefore, it was necessary to study the effect of the preservation space-flight conditions on the viability of algae after being exposed.

These investigations, which were made under the leadership of Professor A. A. Nichiporovich, formed part of the medical and biological experiments made in artificial satellites (Zhukov-Verezhnikov et al., 1961).

Without going into the question of the physical characteristics of cosmic radiation and its possible biological effects, which have been described in detail by Gyurdzhian (1961), work relating to the effect of hard radiation on single-cell algae will be considered.

It is known, from the literature, that single-cell organisms are usually less sensitive to radiation effects than cells of more highly organized forms of life (Alexander, 1959 - Russian translation ). In particular, the very high resistance of various algae to radiation as compared to that of higher plants and animals was observed by Godward (1960), who drew attention to the particularly high resistance of chlorella which survived experimental radiation doses of 1,000,000 rad. However, a number of authors (Gilet, Ozenda, 1960; Musayev, 1961; Schwarze and Frandsen, 1960) observed an appreciable suppression of the growth, breakdown (discoloration) of the chlorophyl and destruction of cells irradiated with doses of gamma and X-rays as low as 20-25 thousand rad. Smaller doses of irradiation (from 1-5 to 20 thousand rad), which produce a temporary stimulation of the growth, also cause considerable changes in the cells.

These are indicated by discoloration and a change in the shape and dimensions of the cells. The production of oxygen during photosynthesis appears to be the property most easily affected by radiation (Zill and Tolbert, 1958).

At radiation doses in excess of 20-25 thousand rad, the reestablishment of the normal rate of growth of the algae is delayed in proportion to the dose of radiation. Thus Gilet (1960) observed that the growth of Scenedesmus cultures was not restored until the 39th day after X-ray irradiation with a total dose of 160,000 rad.

On the other hand, it is known that at low lethal doses of irradiation the cells may not die off immediately but much later as a result of after-effect reactions. Tarusov (1960) has shown that destruction of yeast cells may occur only 3-15 days after $\gamma$-irradiation as a result of after-effect reactions, and this indicates that the lack of observable damage during the first cell divisions cannot be considered as an indication of complete absence of injury. After irradiation with doses of 200 thousand rad and higher, complete destruction of chlorella cells was observed by Musayev (1961) only on the 15-20th day of cultivation.

Thus, as a result of the action of hard radiations and the entire set of space flight conditions, algae cells may be destroyed or their growth suppressed. Partial and reversible depression of photosynthesis may occur. Metabolic processes may also be seriously damaged as the result of changes caused in the rate of photosynthesis, the mechanism of cell division and because of the formation of mutant forms.

Consequently, to elucidate the effects of cosmic radiation and other space flight factors on the algae cells, it was necessary to expose suitable cultures to space-flight conditions and to study these in detail immediately after the flight. It was also necessary to follow for a long period after the flight, the growth and development of the culture as well as the preservation of the genetic properties.

## Characteristics of the Cultures and Experimental Conditions

Chlorella pyrenoidosa was used in the experiments which is considerably more sensitive to the effects of external factors than Chlorella vulgaris (Musayev, 1961). Since it is known (Alexander, 1959) that cells which are actively dividing are more sensitive to radiation, we used in these experiments cultures in the stationary phase of growth - in the form of a "streak" on an agar slant, as well as cultures in a liquid nutrient, taken during the period of their intensive growth. All possible variants of the experiment were performed twice. The culture in the liquid medium, taken at various suspension densities, was poured into glass ampule, which was then sealed into special bags made of a transparent

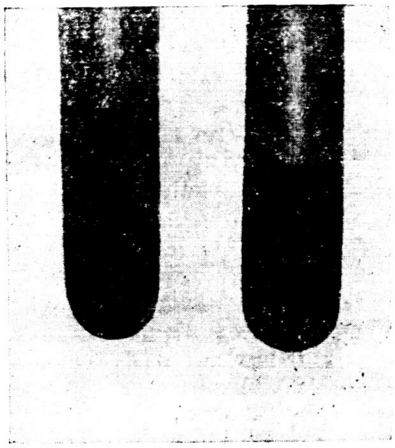

Figure 1. Perspex Ampules Containing Chlorella Cultures on an Agar Slant and in a Liquid Medium

film (Figure 2) so that liquid did not leak into the capsule if an ampule was broken. The ampules were fixed on a rack (Figure 3) which was

Figure 2. Protective Bags Housing the Ampules Containing Chlorella Suspensions

so positioned in the capsule to be catapulted in such a way that the suspension of the algae could be illuminated when taking cinefilms and television recordings in the satellite during the entire period of the test. The cultures on the agar slant and one of the cultures in liquid medium were held in darkness in perspex ampules (Figure 1), and placed in the container together with other microorganisms. Table 1 gives the characteristics of the different cultures used in the experiment.

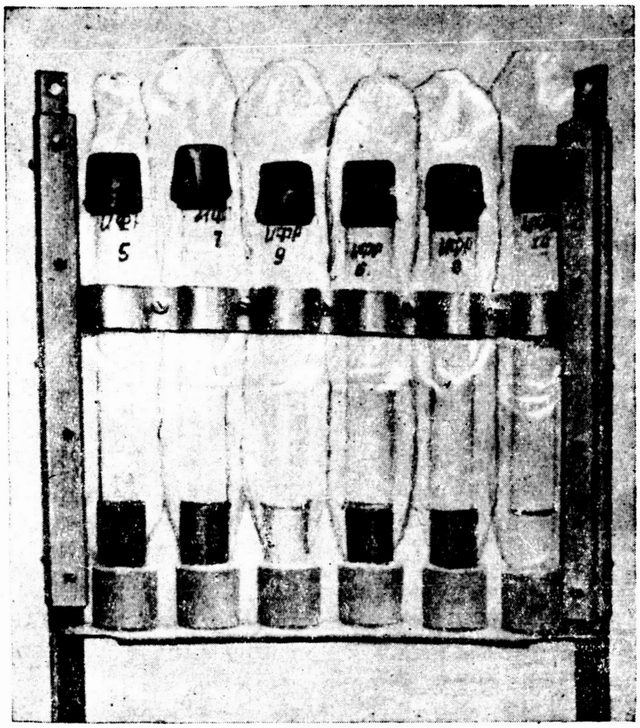

Figure 3. Rack with Ampules Containing Chlorella Suspensions in a Liquid Medium

Ampules containing two sets of control cultures were prepared simultaneously with the experimental cultures. Prior to exposure to space

Table 1. Description of Chlorella Cultures Used in the Experiments

| No. of variant | Sequence No. | Characteristics of the cultures | | |
|---|---|---|---|---|
| | | Ampule | Medium | No. of cells per 1 ml of suspension |
| I | 1<br>2 | Perspex, in darkness | Agar-agar | Stroke |
| II | 1<br>2 | " | Liquid medium | $37.5 \cdot 10^6$ |
| III | 1<br>2 | Glass, exposed to light | " | $232.0 \cdot 10^6$ |
| IV | 1<br>2 | " | " | $37.5 \cdot 10^6$ |
| V | 1<br>2 | " | " | $3.0 \cdot 10^6$ |

conditions, the experimental and control cultures were stored in a refrigerator at 5-10° C. During exposure of the experimental ampules in space, the control ampules were maintained on the ground under identical temperatures, either in darkness or under the same intensity of illumination and for the same periods of time as the experimental ampules.

## Examination of Cultures Which Had Been Exposed to Space-Flight Conditions, Immediately After They Returned to Earth

The state of the algae cells, which had flown in space, was determined immediately after their return to Earth by studying their external characteristics, microscopic appearance and the extent of their photosynthetic activity (as determined by the method of Winkler) compared to that of the control specimens.

Examination of the external state of the culture showed no clumping of cells or the presence of a flaky deposit or other abnormal phenomena which would evidence a diseased culture. A slight etiolation of the suspension compared to the controls took place in cells subjected to the experimental conditions III, IV and V.

The results of microscopic analyses, reproduced in Figure 4a-e, indicate that broken up cells were present in all the experimental cultures but not in the controls. A relatively larger number of broken up cells was detected in liquid cultures of lower density.

## Report on the Microscopic Analyses Shown in Figure 4

(The numbers of the experimental conditions correspond to those shown in Table 1)

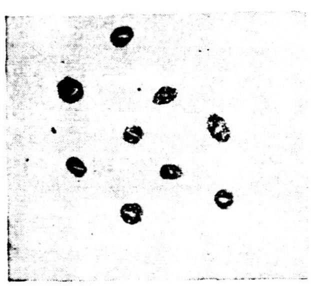

Figure 4. Comparative Morphological Characteristic of the Chlorella Culture after Exposure to Space-Flight Conditions (Table 1)

Figure 4a. Initial Chlorella Culture

a - Initial state of the chlorella culture. Bright green, sharply outlined clear edges, succulent, mainly young, recently divided, with average and somewhat below average dimensions. About 8 to 15 percent large, dividing cells. Pyrenoid clearly outlined.

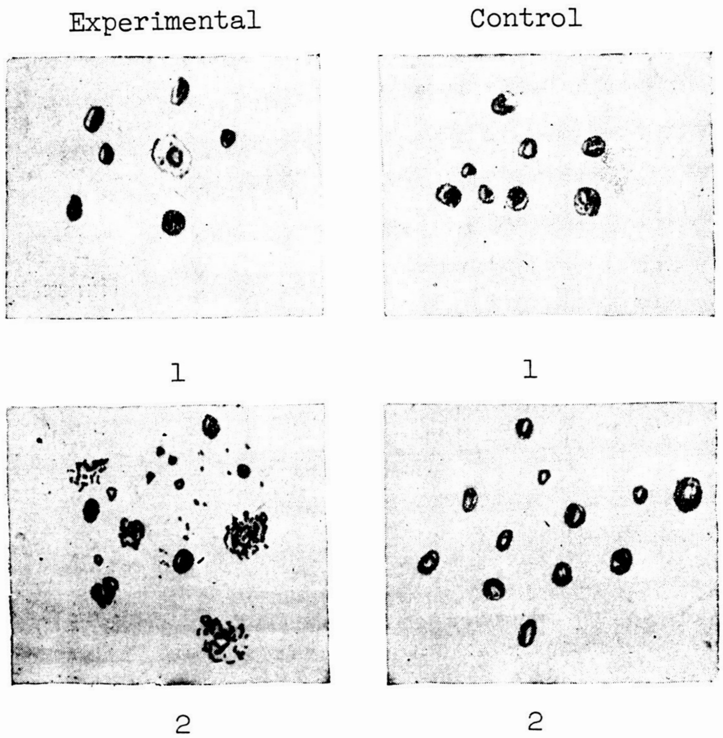


Figure 4b. Condition II

b - Condition II

1st experiment

Experimental - cells mainly of average dimensions, bright green, clear empty vacuolated and broken up cells, and fragments present.

Control - cells bright green with clear cut edges. Pyrenoid present. Cells mainly of average and somewhat larger size, with dividing cells present.

Repetition

Experimental - very many broken up and "spread-out" cells, as well as fragments and protoplasts. Cells without pyrenoid, of average and small dimensions.

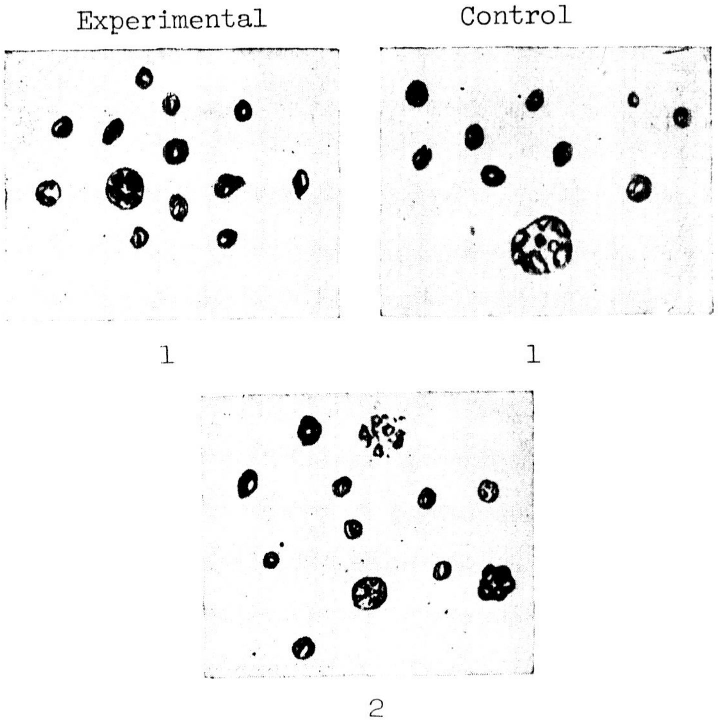


Figure 4c. Condition III

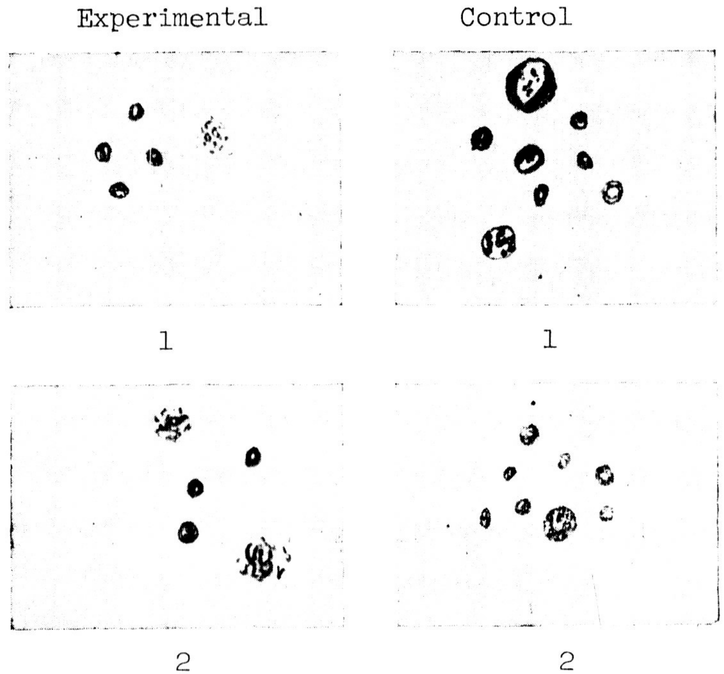


Figure 4d. Condition IV

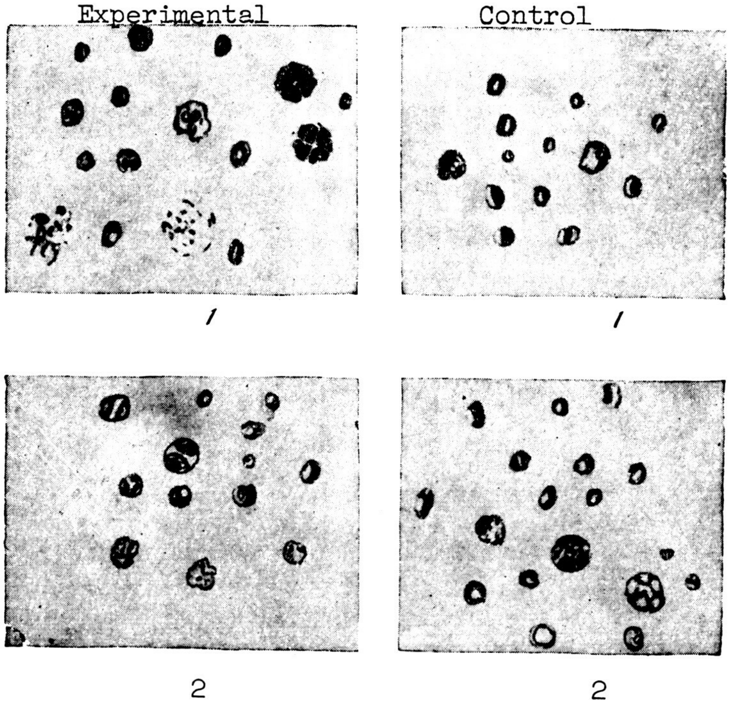


Figure 4e. Condition V

Control - cells mainly of normal dimensions, a few dividing. All the cells bright green with clear cut edges, optically complete. No pyrenoid present. The culture was very uniform.

c - Condition III

1st experiment

Experimental - cells bright green with sharply outlined edges, clear, pyrenoid present but small. The cells were mainly of normal and slightly smaller dimensions. Dividing cells and cells which had just divided present. There were also "loose" spongy cells.

Control - culture uniform with respect to cell dimensions. Cells were mainly young, normal size, bright green, with sharply outlined edges. Cells dividing into four groups and more present.

Repetition

Experimental - cells basically of normal and smaller size. Normal color, edges clearly defined. There were also granular, large cells, and

occasionally broken up cells from which the protoplasm was spreading. Pyrenoid present.

d - Condition IV

1st experiment

Experimental - cells of normal color, young, of normal dimensions with a clearly pronounced pyrenoid and sharply outlined edges; broken up cells were rare but present.

Control - cells of normal color, mainly of normal and smaller dimensions. Occasionally there were large granular cells. Very rare - vacuolated cells.

Repetition

Experimental - the culture was contaminated, many broken up cells and bacteria present. Cells were of small and average dimensions, bright green.

Control - bright green cells of medium and smaller dimensions. The edges were sharply outlined. The pyrenoid was clearly defined. Large granular dividing cells present.

e - Condition V

1st experiment

Experimental - cells of average and slightly larger dimensions, there were many small ones and in most of the cells the edges were "blurred". Many cells granular, broken up and partially broken up from which protoplasm was spreading. Occasionally dividing cells were observed.

Control - cells were bright green, primarily of normal dimensions but there were also smaller and larger ones. A few very large granular cells were present. The edges were sharply outlined. Pyrenoids present. Dividing cells but no broken up cells were observed.

Repetition

Experimental - bright green cells. Division, "mound-shaped" cells present, mainly of normal dimensions. Light green cells also observed.

Control - cells of medium and larger dimensions. Bright green, frequently the chromatophore adjoins the membrane. The edges were sharply outlined. Pyrenoid clearly pronounced. Large granular cells as well as dividing cells present.

Measurement of the photosynthesis in experimental and control cultures by the method of Winkler shows a decrease in the photosynthetic activity of the cells under the experimental conditions (Table 2).

Table 2. Determination of the Intensity of Photosynthesis in the Algae Cells After Exposure in Space, as Compared to the Control

| Experimental condition number | Number of cells per $cm^3$ during exposure according to the Winkler method | | Quantity, $mg/O_2/10^6$ cells for a 2-hour exposure | |
|---|---|---|---|---|
| | Experimental | Control | Experimental | Control |
| I | $0.38 \cdot 10^6$ | $0.26 \cdot 10^6$ | $4.21 \cdot 10^{-3}$ | $1.15 \cdot 10^{-3}$ |
| III | $0.79 \cdot 10^6$ | $0.92 \cdot 10^6$ | $2.28 \cdot 10^{-3}$ | $3.15 \cdot 10^{-3}$ |
| V | $0.21 \cdot 10^6$ | $0.22 \cdot 10^6$ | $1.43 \cdot 10^{-3}$ | $6.82 \cdot 10^{-3}$ |

Exposure to light was made in three periods of two hours each using an illumination of 10,000 lux at the surface of the glass phials.

Thus, under the conditions of space flight, changes were produced in the functional and the morphological states of the chlorella cells which were suspended in a liquid medium during the experiments. Cells which were kept on agar slants remained bright green in color, showed no morphological changes and possessed a high photosynthetic activity.

Studies on the growth of algae exposed to space flights and the availability of methods for detecting mutant forms should provide an answer to the question of the importance of the factors of cosmic flight on viability and the basic physiological functions of chlorella.

## Study of the Growth of Chlorella in Culture Following Exposure to Space Flights

Experiments on growing cultures of chlorella were carried out under conditions which insure intensive growth of these plants (Vladimirova and Semenenko, 1962). The cells were grown in suspension in a liquid mineral medium into which carbon dioxide-enriched air was blown (Figure 5). The tubes of growth medium were inoculated with the same number of cells despite the different experimental treatments which these had undergone. Therefore, control and experimental specimens treated in the same way

could differ only in the number of viable and not in the total number of cells initially inoculated.

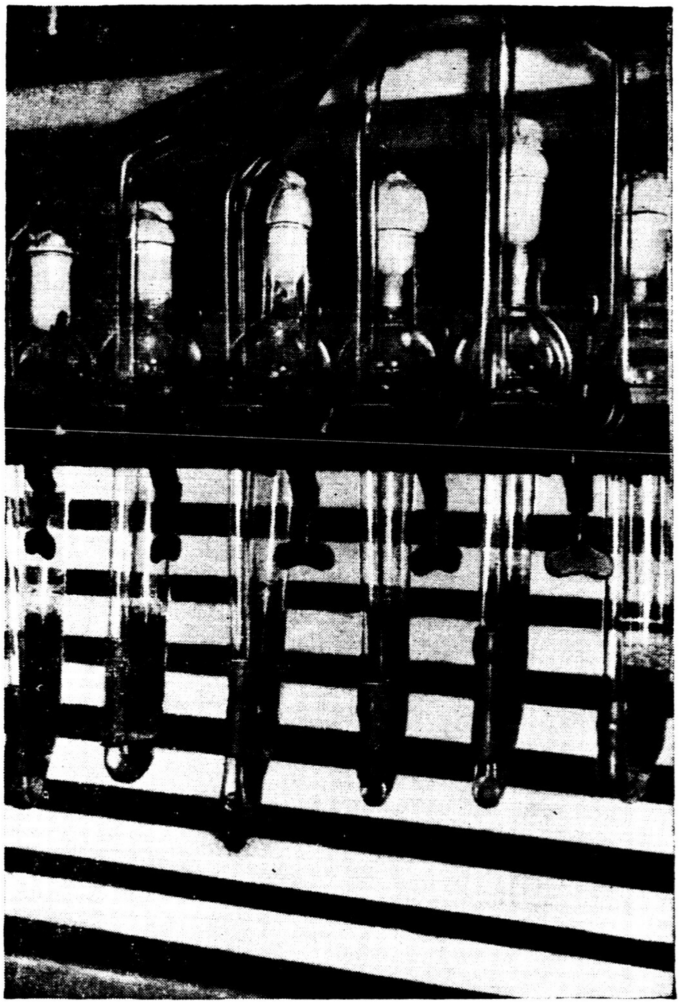

Figure 5. Vessels for Growing Algae under Conditions Insuring Rapid Growth

The progress of the growth of the culture was determined by counting the number of cells under a microscope and also by measuring the density of the culture in a nephelometer. At the end of the experiment the dry weight of the culture and the rate of photosynthesis of the chlorella cells were determined. The results of the experiments are given in Table 3, Figure 6 and Table 4.

The kinetics of growth, the accumulation of organic matter, morphological characteristics, dimensions and the dry weight of the cells as well as the generation of oxygen during the photosynthesis in the experimental and control cultures are the same, within experimental error. An exception to this is the low rate of photosynthesis of cells grown under condition V. The growth characteristics of these did not repeat satisfactorily (Figure 6, V).

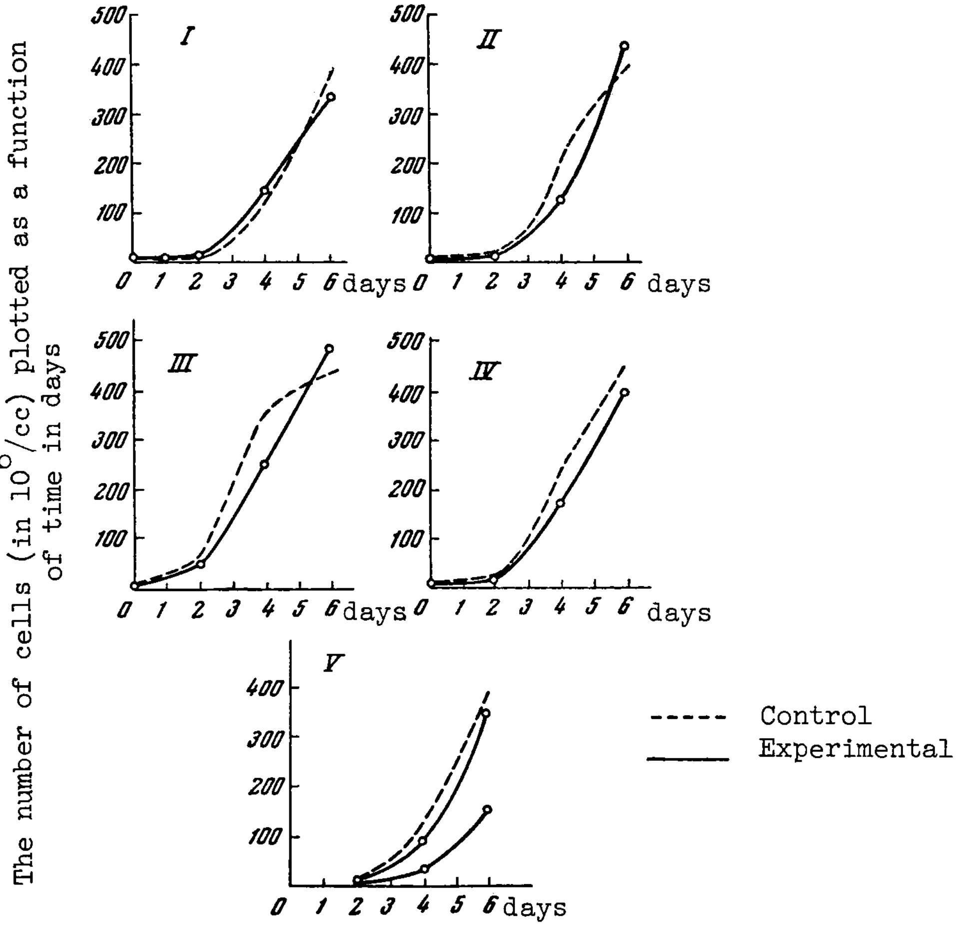


Figure 6. Growth Curves of Experimental and Control Cultures of Chlorella after the Experimental Cultures were Exposed to Conditions of Space Flight
Roman numerals: experimental conditions

The results obtained lead to the conclusion that the differences between the algae cultures which were flown for 24 hours in the satellite and their respective controls observed during the first examination almost entirely disappeared when the algae were cultivated under conditions of rapid growth.

Table 3. Comparison of the Rates of Growth of Experimental and Control Specimens of Chlorella Pyrenoidosa

| Experimental condition number | Specimen | Number of cells with the progress of growth of the culture, $10^7$ ml | | | |
|---|---|---|---|---|---|
| | | Inoculation | 2nd day | 4th day | 6th day |
| I | Experimental | 0.46 | 4.37 | 143.7 | 338.2 |
| | Control | 0.31 | 2.40 | 125.0 | 382.5 |
| II | Experimental | 0.67 | 12.85 | 124.7 | 437.5 |
| | Control | 0.61 | 13.90 | 206.0 | 383.7 |
| III | Experimental | 6.40 | 50.80 | 255.3 | 494.0 |
| | Control | 12.00 | 66.90 | 364.5 | 438.5 |
| IV | Experimental | 1.55 | 16.85 | 169.8 | 398.5 |
| | Control | 1.97 | 27.20 | 243.9 | 468.3 |
| V | Experimental | 0.29 | 1.60 | 92.5 | 355.0 |
| | Control | 0.43 | 4.45 | 137.5 | 385.0 |

The view that no important irreversible changes occur in chlorella cultures is supported by an attempt which was made to detect mutant cells. To search for these, samples of experimental and control specimens were poured in soft agar into a Petri dish containing a mineral medium agar ($1^{\circ}$ Blg.). No visible changes could be detected in the colonies which would distinguish them as being mutants. All colonies of algae were found to be uniform in color, shape and dimensions. Identical results were obtained by us in later experiments on the same strain of chlorella.

According to Bulbon (1961), no changes were detected in algae in experiments carried out in November, 1960, in the "Discoverer-17" satellite in the U.S.A.

## Conclusions

1. The culture of Chlorella pyrenoidosa, which was flown in the second Soviet satellite for a period of 24 hours, was not subject to destructive factors during the launching, flight or landing of the satellite, it maintained its viability and did not reveal irreversible changes in the basic physiological processes of photosynthesis, growth, development and reproduction.

2. Immediately after return to Earth, the cultures displayed a reduced rate of photosynthesis and a considerable number of broken down

Table 4. Comparison of Experimental and Control Specimens of Chlorella Expressed in Terms of Number of Cells, Dry Weight and Rate of Photosynthesis (based on $O_2$ generation)

on the Sixth Day of Cultivation

| Experimental condition No. | Density of suspension $10^6$ cells/ml | | | | Dry weight, mg | | | | Rate of photosynthesis, $mgO_2$ per $10^6$ cells | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Initial | | At the end of the experiment | | Per 1 $cm^3$ suspension | | Per $10^8$ cells | | | |
| | Experimental | Control | Experimental | Control | Experimental | Control | Experimental | Control | Experimental | Control |
| I | 0.46 | 0.31 | 338.2 | 382.5 | 3.8 | 3.7 | 1.1 | 1.0 | $5.3 \cdot 10^{-3}$ | $5.3 \cdot 10^{-3}$ |
| II | 0.67 | 0.61 | 437.5 | 383.7 | 4.3 | 4.6 | 1.0 | 1.2 | - | - |
| III | 6.4 | 12.2 | 494.0 | 438.5 | 5.6 | 5.7 | 1.1 | 1.3 | $10.4 \cdot 10^{-3}$ | $11.9 \cdot 10^{-3}$ |
| IV | 1.55 | 1.97 | 398.5 | 468.3 | 4.7 | 4.8 | 1.2 | 1.1 | - | - |
| V | 0.29 | 0.43 | 355.0 | 385.0 | 4.4 | 4.8 | 1.2 | 1.2 | $1.2 \cdot 10^{-3}$ | $9.2 \cdot 10^{-3}$ |

cells were observed. However, after six-day cultivation under active growth conditions the exposed cultures behaved in the same way as control cultures grown under normal conditions.

Summary

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Cultures of Chlorella pyrenoidosa in sealed ampules in liquid nutrient media of varying densities and on agar slants were exposed to the effects of space flight for 24 hours on the second orbital spaceship. Periodic illumination was provided for photographic and television observations. Macroscopic observations of the cultures immediately upon return to Earth did not reveal clotting, cottony deposits, or other pathogenic signs. Comparative studies of cell morphology, photosynthetic activity, mutations, and culture growth and development all indicated that the experimental cultures had good resistance to the effects of launching, space flight, and reentry. In general, viability was preserved and the principal physiological processes, i.e. photosynthesis, growth, development and reproduction, showed no irreversible changes. However, the cultures had varying amounts of damaged cells and upon cultivation showed decreased photosynthetic activity immediately after landing. After six days of active growth the algae approached the normal condition of the control cultures.

Author

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# EFFECTS OF FLIGHT IN THE SECOND SOVIET SATELLITE ON THE HEMOPOIETIC ORGANS OF ANIMALS

M.A. Arsen'yeva, V.V. Antipov, V.G. Petrukhin, T.S. L'vova
N.N. Orlova, S.S. Il'ina

Numerous data have accumulated in the world's literature on changes produced in various organs and tissues by irradiation, e.g. $\alpha$, $\beta$,$\gamma$-rays and neutrons.

Nothing is known, however, as to the effect of other radiations, e.g.,cosmic rays which consist of high energy particles. There are no data on the biological effect of cosmic rays and other factors in cosmic space travel on living organisms.

The present paper is the first attempt to study the effect of a cosmic flight on the hemopoietic organs of animals. For this purpose laboratory mice were included among other living subjects in the second satellite flight.

The test series comprised 40 inbred black (C-57) and unrelated white mice. A second group of the same number, kept under identical living and feeding conditions, but which did not take part in the flight, served as controls. The mice returning from the flight were found to be alive and in perfectly good general condition.

Cyto-histological observations were made of the bone marrow, spleen and peripheral blood.

The mice were sacrificed 2, 3, 5, 9, 30 and 60 days after returning from the flight. The controls were sacrificed at the same time the peripheral-blood tests were taken from the tip of the tail in all cases.

The complete bone marrow used for the cytological tests was fixed in a Carnoy's fluid and stained with acetocarmine. The bone marrow cells were examined cytologically for the presence of possible mitotic disturbances induced by the flight or by cosmic rays.

The various mitotic disturbances caused by radiation are associated with structural changes in the chromosome, known as "chromosome rearrangements". As these are easily observable the effects of radiation on the cell nucleus can be studied. As a rule this chromosome rearrangement disturbs the normal chromosome arrangement in the nucleus and so directly causes tissue lesions.

Analysis of the bone marrow cells taken from the test animals and controls included observation of the normal and damaged cells in anaphase

and telophase chromosomal abnormalities appearing as typical bridges, chromosomal fragmentation and adhesions which produced more apparent bridges.[1]

The frequency of anaphase and telophase disturbances in bone marrow cells was analyzed in 24 test animals and 10 controls. In both groups the percentage of chromosome abnormalities showed considerable individual fluctuations. For the controls, the percentage of animals showing changes varied between 1.96 and 3.8 percent the mean frequency of mitotic disturbance being 3.16 ± 0.42 percent. In all cases the observed individual percentage fluctuations of mitotic disturbances among the test animals exceeded those for the controls. The test results are given in Table 1.

The data of Table 1 indicate in the comparison of the bone marrow cells of test animals with those of the controls, that there is a statistically greater probability of mitotic disturbances occurring among the test animals.

Before considering the data any further it should be borne in mind that animals like rats and mice, exposed to general ionizing radiation show a decrease of mitotic disturbances after a given number of days, depending on the radiation dose. This even applies to animals given lethal doses which showed towards the end of the fifth week or even earlier a decrease to the control level (Devik, 1954; Devik and Lothe, 1955; Dubinin, Arsen'yeva and Kalyayeva, 1962).

After irradiation with 400 r the bone marrow cells showed about 30 percent chromosomal re-arrangement on the 2nd day. On the 5th day after irradiation this percentage decreased to 22 percent, on the 10th day to 18 percent and on the 30th day to 2.6 percent, i.e.,to control level. With a dose of 50 r the percentage of chromosomal re-arrangement reached 11 percent, falling to 4 percent after only 10 days. In one respect the data on chromosomal abnormality during the mitosis of the bone marrow cells of mice after a space flight differ from the X-ray data. As shown in Table 1, the frequency of chromosomal abnormality in bone marrow cells reached 7.12 percent on the second day; 60 days after the flight it was practically unchanged - 7.63 percent. During this period of 8 weeks no decrease was observed and by the 30th day there was even some, if slight, increase in the frequency of chromosomal abnormality to 11 percent.

We will now consider the types of chromosomal abnormality occurring in bone marrow cells after a space flight. The following points are

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[1]The material obtained from vibration tests, as explained below, was subjected to a special analysis for changes producing chromosomal adhesions.

Table 1. Frequency of Chromosome Disturbances in Blood-Forming Cells of the Bone Marrow of Mice After a Space Flight

| Sacrificed after days | Number of test animals | Number of normal anaphase and telophase stages | Number of disturbed anaphase and telophase stages | Total cells tested | Mitotic disturbance, percent | Mean percentages of mitotic disturbances in 10 controls sacrificed at different times | $M_{diff} \pm m_{diff}$ | Ratio |
|---|---|---|---|---|---|---|---|---|
| 2nd | 4 | 887 | 68 | 955 | 7.12±0.83 | 3.16±0.46 | 3.96±0.95 | 4.1 |
| 3rd | 3 | 460 | 42 | 502 | 8.36±1.23 | | 5.20±1.31 | 3.9 |
| 5th | 4 | 620 | 53 | 673 | 7.88±1.03 | | 4.72±1.13 | 4.1 |
| 9th | 5 | 1790 | 155 | 1945 | 7.96±0.56 | | 4.80±0.72 | 6.6 |
| 30th | 4 | 637 | 77 | 714 | 10.78±1.16 | | 7.62±1.24 | 6.1 |
| 60th | 4 | 750 | 62 | 812 | 7.63±0.95 | | 4.47±1.03 | 4.3 |

evident even on cursory examination. The effect of γ-rays or neutrons on bone marrow cells, even with a small dose of 50 r, is always to produce a considerable number of bridges with fragmentation and anaphases with fragmentation. In this case we are dealing with true chromosome re-arrangements (Dubinin, Arsen'yeva and Kalyayeva, 1960). Analysis of the data on mice obtained after the flight shows that the percentage of bridges with fragmentation and of anaphases with fragmentation is appreciably lower than that produced by only one irradiation. In Figures 1, 2, a, b, c; 3, a, b, c, d are shown microphotographs of the types of chromosome disturbances found with the mitotic figures of bone marrow cells after only one irradiation, in satellite flight tests, and in vibration tests. Figure 1 is a microphotograph of a normal late anaphase.

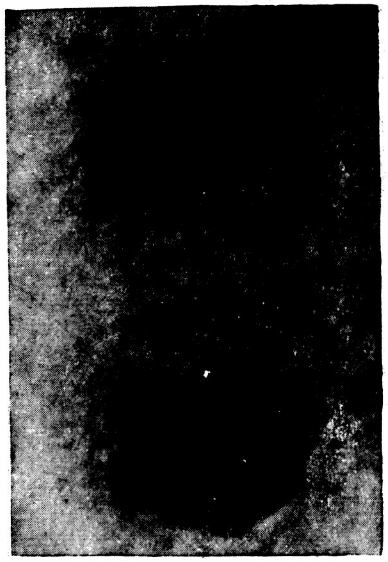

Figure 1. Normal Retarded Anaphase in Bone Marrow Cells of Mice. 900X

A comparison of the data on the frequency of the different chromosomal abnormalities occurring in bone marrow cells of mice two days after the flight with data on the effect of X-rays (Table 2) shows that doses of 50 r produced by the 2nd day 6.67 percent chromosomal re-arrangement (bridges), 4.44 percent bridges with fragmentation, and a number of anaphase stages. In the flight test the total percentage of chromosomal abnormalities was almost the same as with a dose of 50 r, but only four anaphases in all were found showing chromosome fragmentations, i.e.,0.42 percent. The percentage of fragmentation occurring in the controls was 0.30 percent.

Thus, cosmic flight differs in its effect in two fundamental respects from that of radiation under terrestrial conditions: First - there was no decrease in the frequency of chromosomal abnormalities occurring

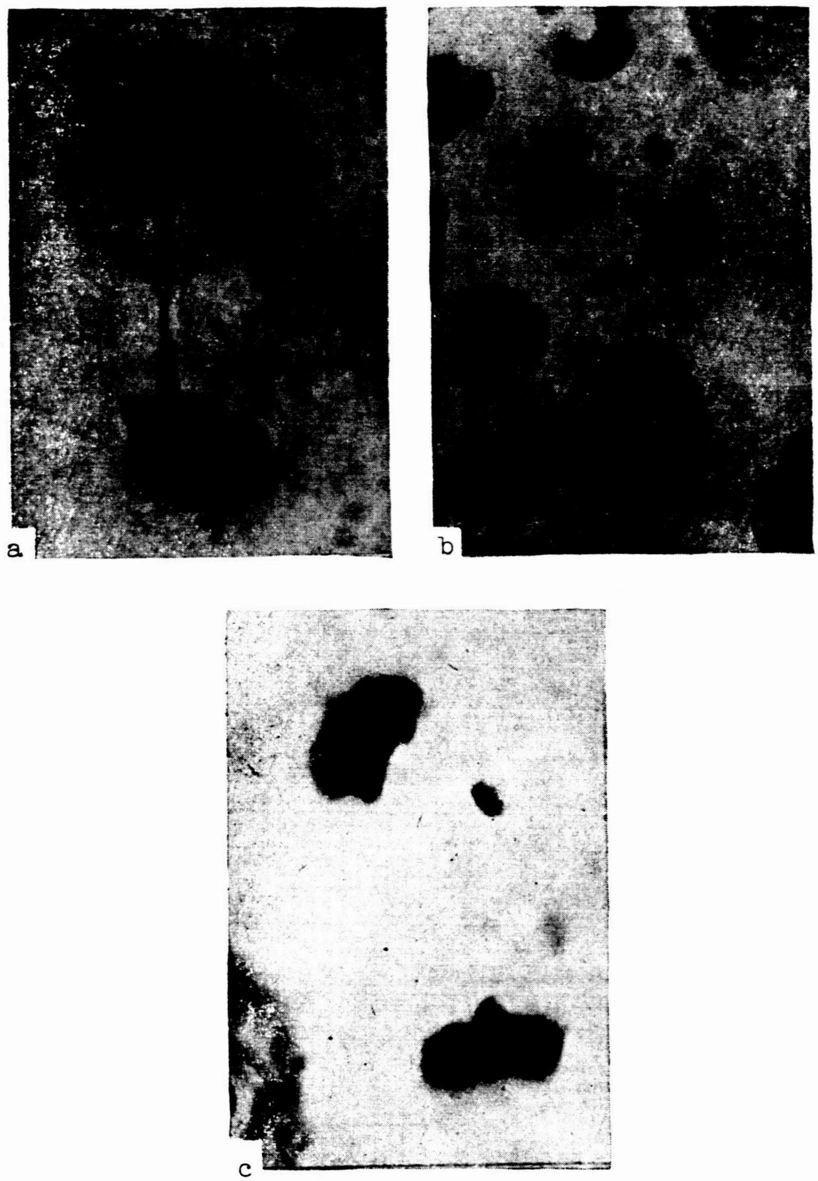

Figure 2. Types of Chromosome Disturbances Found After Treatment With Ionizing Radiation. a, b- chromosome bridges with fragmentation, 900X; c- telophase with fragmentation, 900X

between the onset of the effect and the end of the test; second, chromosomal abnormalities induced by cosmic flight and with a frequency corresponding to that of the chromosome re-arrangements produced by a dose of X-rays of 50 r, are characterized by a practically total absence of chromosome fragmentation. Moreover, the nature of chromosomal abnormalities caused by a flight seems to indicate that possibly most of them are

due not to breaks, but to adhesion and subsequent incurred separation (Darlington and LaCour, 1952).

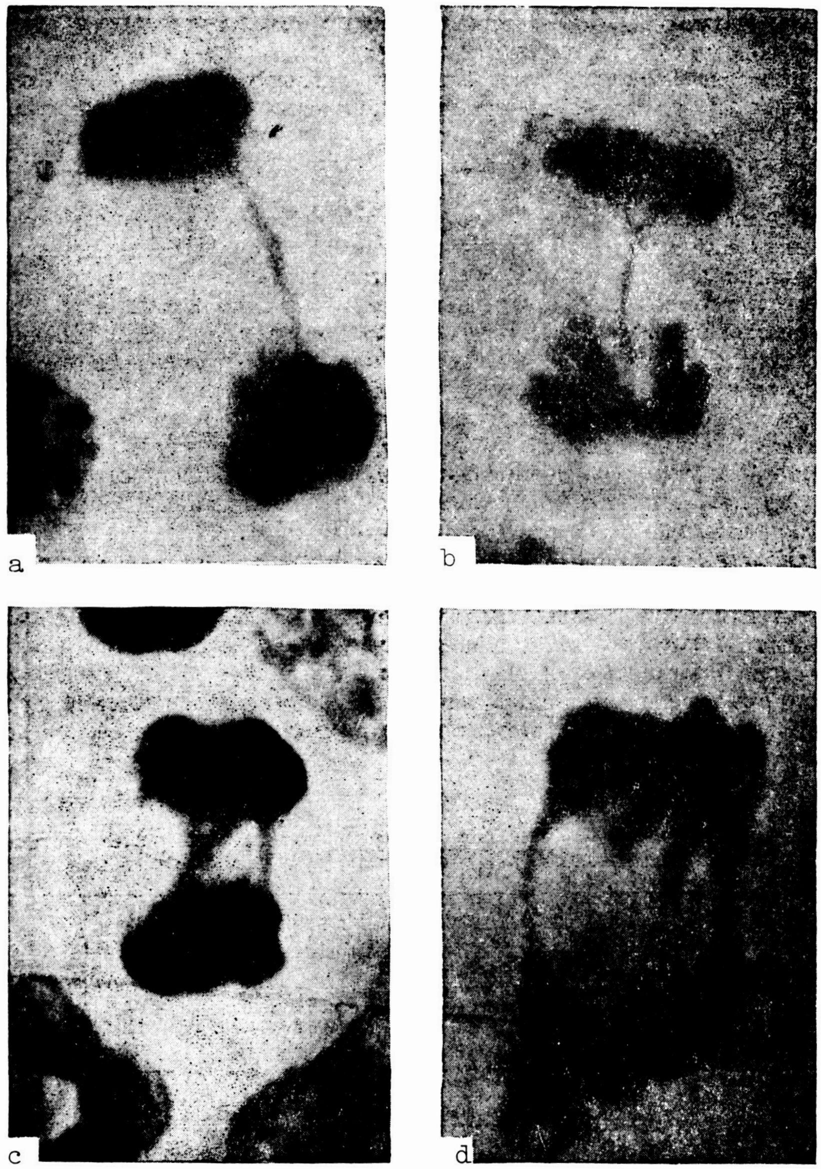

Figure 3. Type of Chromosome Disturbances Found After a Satellite Flight and Exposure to One Vibration Treatment. a, b- chromosome bridges, 900X; c, d- adhesion of chromosomes and formation of apparent bridges, 900X

We will now deal with the morphological bone marrow picture. In mice sacrificed on the 30th day after return to Earth we found considerable white cell proliferation, as evidenced by an increase in the

myeloblast, premyelocyte and myelocyte count. Moreover, numerous young eosinophils were evident, with characteristic differentiation of the nucleus and protoplasm. The red cell count was very low.

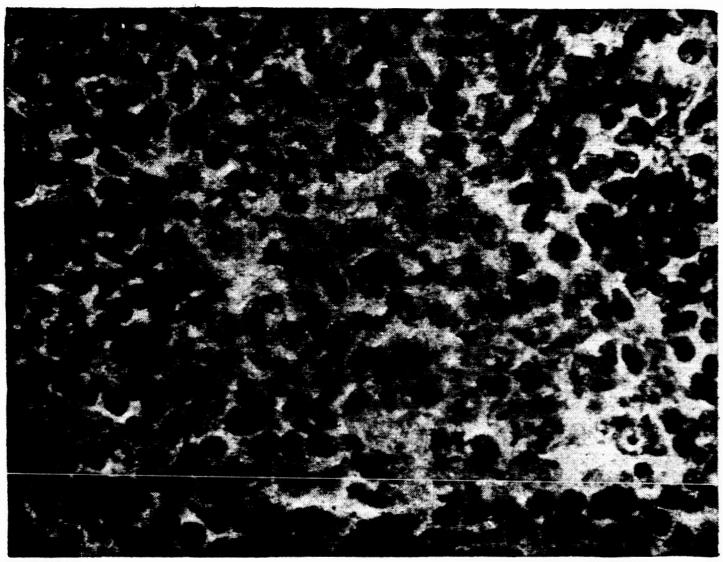

Figure 4. Spleen of Mouse Sacrificed Three Days After the Flight. Center of lymphatic follicle destroyed, consisting mainly of reticular cells, 900X

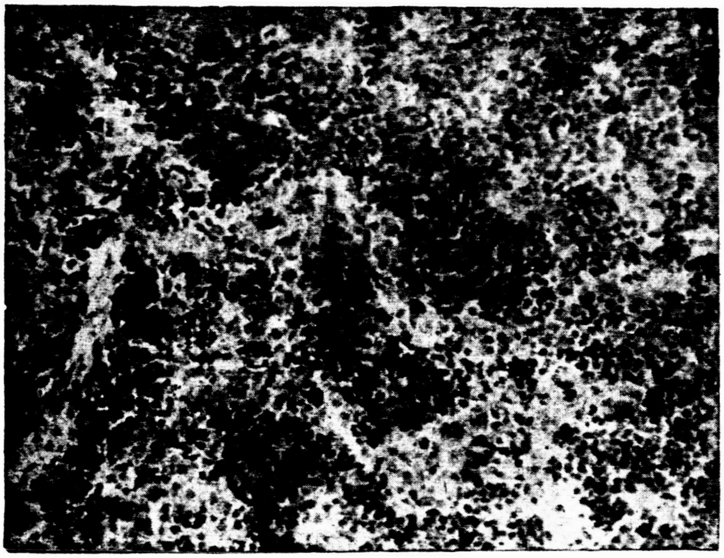

Figure 5. Spleen of Mouse Sacrificed 30 Days After the Flight. The entire organ contains amorphous hemosiderin granules. No recognizable follicle boundaries, 280X

Table 2. Frequency of Chromosomal Abnormality in Bone Marrow Cells of Mice on 2nd Day After the Flight and After an X-ray Irradiation Test

| Cause | Number of normal anaphases and telophases | Anaphases and telophases with bridges | | Anaphases and telophases with fragmentation and bridges | | Anaphases and telophases with fragmentation | | Total anaphases and telophases disturbed | | Total cells examined |
|---|---|---|---|---|---|---|---|---|---|---|
| | | number | percent | number | percent | number | percent | number | percent | |
| Space flight | 887 | 64 | 6.70 | - | - | 4 | 0.42 | 68 | 7.12 | 955 |
| X-rays (dose 50 r) | 200 | 15 | 6.67 | 5 | 2.22 | 5 | 2.22 | 25 | 11.1 | 225 |

The peripheral blood picture of the test animals after the flight shows, however, no appreciable changes when compared with the controls (Table 3).

Preliminary histological examination by the usual staining methods (hematoxylin-eosin, scarlet red, Romanovsky-Giemsa) shows a lower megakaryocyte count in the spleen of mice sacrificed on the 3rd day after the flight. The center of lymphatic follicles consist in part of "pale" areas made up mainly of reticular cells (Figure 4). By the 9th day the follicles decreased in size and the protoplasm of the reticulo-endothelial cells contained distinct hemosiderin granules. By the 30th day the histological aspect of the spleen had changed. The follicles were enlarged, with indistinct boundaries and contained cells of different shapes, sizes and color (Figure 5). Among these are numerous larger, hyperchromatic cells resembling young myeloid type cells (Figure 6).

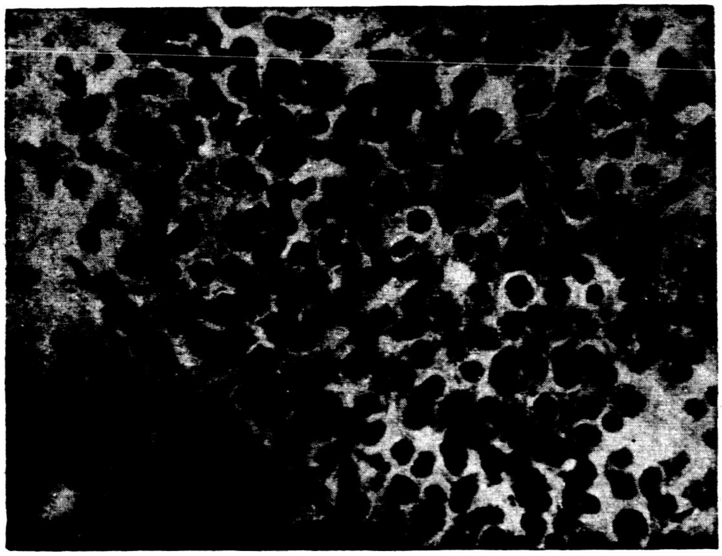

Figure 6. Spleen of Mouse Sacrificed 30 Days After the Flight, Showing Polymorphism of the Peripheral Cells of the Follicle, 900X

These myeloid cells are also found outside the follicles. At about the same time a slight increase of the megakaryocyte count was observed. Hemosiderin granules occurred more frequently in the cytoplasm of the reticulo-endothelial cell.

During the first days of the test (up to the 10th day) disturbances of lympho- and myelopoiesis occurs, but by the 30th day the hematopoietic activity of the spleen is restored and new young cells appear.

Table 3. Peripheral Blood Pic-

| Test No. | Group | No. of animals | Tested after days | Leucocyte count | Neutrophils M | Neutrophils 1* | Neutrophils 2* | Neutrophils 3* |
|---|---|---|---|---|---|---|---|---|
| 1 | Test | 6 | 2nd | 6,850-19,600 | - | 0.5-2.5 | 2.5-6 | 10-46.5 |
| 2 | Test | 5 | 3rd | 6,050-19,000 | - | 0.5-2 | 2-7.5 | 15-41.5 |
| 3 | Control | 5 | " | 6,500- 9,300 | - | 0.5-1.5 | 1-5.5 | 7-53 |
| 4 | Test | 6 | 5th | 9,150-12,050 | - | 0.5-2 | 0.5-8.5 | 6-44 |
| 5 | Control | 6 | " | 6,200-19,050 | - | 0.5-1.5 | 1-6.5 | 8-54 |
| 6 | Test | 5 | 9th | 7,995-17,400 | - | 0.5-2.5 | 2.5-6 | 10-46.5 |
| 7 | Control | 5 | " | 7,580-12,650 | - | 0.5-3 | 2-12 | 10.5-40 |
| 8 | Test | 6 | 30th | 6,150-13,000 | - | 1-2 | 0.5-6.5 | 5-47 |
| 9 | Control | 6 | " | 9,950-21,850 | - | 0.5-2 | 1-7.5 | 5-46 |
| 10 | Test | 5 | 60th | 7,350-16,850 | - | 1-2.5 | 0.5-7 | 7-45 |
| 11 | Control | 5 | " | 14,100-18,000 | - | 0.5-2.5 | 1-6 | 5-46 |

*1- Spherical nucleus 2- Stab nuclear 3- Segments nuclear

Table 4. Frequency of Chromosomal
Nuclei of Mice after

| After sacrifice | No. of test animals | Total No. of cells tested | No. of normal anaphases and telophases | No. of disturbed anaphases and telophases: Chromosomal abnormalities | No. of disturbed anaphases and telophases: Chromosomal adhesions | No. of disturbed anaphases and telophases: Total No. of mitoses affected | Proportion of anaphases and: Chromosomal abnormalities | Proportion of anaphases and: Chromosomal adhesions |
|---|---|---|---|---|---|---|---|---|
| 30 min | 3 | 759 | 622 | 61 | 76 | 137 | 8.04±0.98 | 10.01±1.09 |
| 1 hr | 2 | 638 | 489 | 43 | 106 | 149 | 6.74±0.99 | 16.61±1.47 |
| 1 day | 2 | 817 | 571 | 80 | 166 | 246 | 9.79±1.04 | 20.31±1.40 |
| 2 days | 2 | 521 | 404 | 50 | 67 | 117 | 9.60±1.28 | 12.85±1.83 |
| 30 days | 2 | 405 | 356 | 19 | 30 | 49 | 4.69±1.09 | 7.41±1.30 |
| Control* | | 574 | 526 | 15 | 33 | 48 | 2.61±0.69 | 5.75±0.97 |

*Means of data for six intact mice

ture of Test Animals and Controls

| Eosinophils | Monocytes | Baso-phils | Lymphocytes | Erythrocyte count, thous. | Hemoglobin |
|---|---|---|---|---|---|
| 0.5-2.5 | 0.5-4.5 | - | 43-84.5 | 10,700- 9,460 | 13.6-14.4 |
| 0.5-2.5 | 1-4 | 1 | 41.5-83 | 8,280- 9,100 | 13.6-14.6 |
| 0.5-6 | 2-5 | - | 32-81.5 | 8,100-12,350 | 13.4-14.4 |
| 1-4 | 0.5-6 | 2 | 38-84 | 7,390- 9,390 | 13.2-14.0 |
| 0.5-10 | 2-6 | 1 | 28-84.5 | 7,320-10,450 | 12.8-13.6 |
| 0.5-2.5 | 0.5-4.5 | - | 40-83.5 | 8,010- 9,910 | 12.4-15.0 |
| 0.2-2.5 | 1-2 | - | 43-83.5 | 7,990- 8,560 | 11.8-14.6 |
| 0.5-4.5 | 0.5-5 | - | 33-80.5 | 7,950-11,760 | 12.2-15.4 |
| 0.5-6 | 1-4 | - | 33-84 | 9,980-13,880 | 14.2-16.0 |
| 0.5-3 | 1-7 | - | 30-84.5 | 7,900-13,220 | 12.0-16.8 |
| 0.5-2.5 | 0.5-6 | - | 33-84.5 | 7,500-10,990 | 12.0-15.2 |

Abnormalities in Bone Marrow Cell
Exposure to Vibration

| disturbed telophases | Chromosomal disturbances | | Chromosomal adhesions | | Mitotic changes | |
|---|---|---|---|---|---|---|
| Total abnormalities | Difference between tests and controls | Probability factor | Difference between tests and controls | Probability factor | Difference between tests and controls | Probability factor |
| 18.05±1.39 | 5.43±1.18 | 4.6 | 4.26±1.46 | 2.9 | 9.69±1.73 | 5.6 |
| 23.35±1.67 | 4.13±1.19 | 3.4 | 10.86±1.76 | 6.2 | 19.99±1.96 | 7.6 |
| 30.11±1.60 | 7.18±1.24 | 5.8 | 14.56±1.71 | 8.5 | 21.75±1.96 | 11.1 |
| 22.45±1.83 | 6.99±1.45 | 4.8 | 7.10±1.76 | 4.0 | 14.09±2.14 | 6.6 |
| 12.09±1.63 | 2.08±1.29 | 1.6 | 1.66±1.62 | 1.0 | 3.73±1.29 | 2.8 |
| 8.36±1.15 | | | | | | |

The thymus shows much less change; up to the 9th day cell mitosis is much decreased but it increases again by the 30th day.

According to the cytological and histological data hematopoietic organs of mice show definite changes after the flight. As the total dosage of cosmic rays recorded by the physical dosimeters during the flight of the second satellite was insignificant (abt. $10^{-2}$ rad), it may be assumed that the changes in question are due not only to cosmic rays but also to other flight factors, e.g., vibration and acceleration.

In order to differentiate between these factors, tests were carried out to elucidate the effect of vibration and acceleration on the hematopoietic organs of mice. The vibration test conditions were: frequency 70 c/s, amplitude 0.4 mm, exposure time 15 min. Total vibro-overload 10 g. The animals were subjected to an overload of 10 g for 15 min. in the centrifuge. 40 mice were used for the test.

The first group of mice was sacrificed 30 and 60 min. after the test and subsequent groups after 2, 3, 5, 9, 30 and 60 days. Controls were sacrificed at the same times. Killing, tissue fixation and further histological processing was done by the same methods as in the flight test. Blood samples were taken from the tail tips before the mice were killed.

The cytological results of the first vibration tests are analyzed in Table 4.

The increase in the number of chromosomal abnormalities of anaphase disturbances occurring mainly as chromosomal adhesions, is very striking compared with the controls. Normally the percentage of adhesion chromosomes is 5.7, but within 30 min. after exposure to vibration it rises to 10, within 1 hour to 16 and within 24 hours to 20. Thereafter the number of abnormal mitotic stages decreases, but even after 30 days the percentage of abnormalities (7.4) remains higher than the control level.

The number of anaphases and telophases with chromosome abnormalities after 30 min. vibration treatment (8.04 percent) is greater than normal (2.61 percent) and reaches a maximum on the first day (13.44 percent). On the second day this percentage dropped to 9.6. It should be borne in mind that in the flight test the percentage of chromosome disturbances was 7.12 on the second day.

A characteristic of chromosomal abnormality in the vibration test, as in the flight test, is the very low percentage of fragmentation. During the first 24 hours after the vibration test the percentage of chromosome disturbances (bridges only) was 17.40, but the percentage of fragments, and bridges with fragments was 0.98 in all. On the second

day the percentage of chromosomal bridges was 10.13, that of bridges with fragments and of anaphase stages with fragments was 0.88 percent. The percentage of breakages in the controls was 0.30 percent. Comparison with the effects of ionizing radiation shows that an X-ray dose of 100 r produced during the first 24 hours 7.37 percent bridges and anaphase stages with chromosome fragments in the bone marrow cells of mice. The percentage of bridges without fragments was 9.54 percent (Table 5).

The identification of these chromosomal abnormalities is somewhat difficult, hence some of them observed in the vibration tests may not have been true exchanges but caused by the adhesion of fragmented chromosomes.

Cytological analysis of the spleen of a mouse sacrificed 24 hours after the vibration test, also shows the presence of chromosomal adhesions (up to 20 percent) with a total frequency of chromosomal abnormalities of 9.09 percent (control 2 percent). This organ also showed some slight mitotic activity.

Morphological analysis of the bone marrow of mice after the vibration test resulted in the following findings:

After 30 and 60 min. myelopoiesis slowly recommenced with increased myeloblast, premyelocyte and hemocytoblast count. No change was observed in erythropoiesis.

Considerable proliferation of white cells was evident in the bone marrow after two days: numerous myeloblasts and premyelocytes were seen. The number of mitoting myeloblasts increased.

No proerythroblasts were present in the myelogram. After 30 days the myelogram showed no considerable deviations from the normal.

According to the cytological data, chromosomal adhesions are the principal characteristic of the nuclear disturbances produced by vibration. Acceleration tests show that after 30 min. this factor produces similar changes but to a much smaller extent. This fact and also analogous flight phenomena (constant percentage of chromosomal abnormalities, absence of fragmentation and prevalence of chromosome adhesion) indicate that vibration as a mechanical factor is one of the principal causes of chromosome abnormalities during a space flight.

Further careful investigation will be necessary to elucidate the mechanism of the action of vibration on the cell nucleus.

From the data on mice after a space flight and on animals after a vibration and acceleration test under laboratory conditions, it seems likely that the observed changes in the hematopoietic organs (increased

Table 5. Frequency of Different Chromosomal Abnormalities in the Bone Marrow Cells of Mice During the First 24 Hours in the Vibration and X-Ray Exposure Test

| Cause | Number of normal anaphases and telophases | Number of normal anaphases and telophases with bridges | | Number of normal anaphases and telophases with bridges and fragments | | Number of normal anaphases and telophases with fragments | | Total number of disturbed anaphases and telophases | | Total cells examined |
|---|---|---|---|---|---|---|---|---|---|---|
| | | number | percent | number | percent | number | percent | number | percent | |
| Vibration | 333 | 71 | 17.53 | - | - | 4 | 0.99 | 75 | 18.52 | 405 |
| X-rays (dosis 100 r) | 270 | 31 | 9.54 | 6 | 1.84 | 18 | 5.53 | 55 | 16.92 | 325 |

frequency of chromosome abnormalities in mitosis, proliferation of bone marrow and spleen cells) are due largely to the effect of vibration. However, we know nothing about the biological effectiveness of cosmic rays and other flight factors. The problem of the overall effect of the different flight factors, including cosmic rays, cannot as yet be considered as fully solved.

SUMMARY

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This investigation is the first attempt to study changes in hemopoietic organs induced by space flight. Laboratory mice were used in the second satellite flight. Cytological investigations of bone marrow cells in the tested animals showed a statistically significant increase of mitotic damage, compared with the control animals. Comparison of this data with that obtained after exposure of the bone marrow cells to radiation of 50 r indicates that the damage caused by exposure to space flight does not decrease until the 60th day after it. Furthermore, the chromosomal fragmentation so characteristic of radiation damage during chromosome exchanges was almost absent. The morphological picture of bone marrow up to the 30th day after flight revealed an increase of myelopoesis accompanied by the appearance of young white blood cell forms and an inhibition of erythropoesis. The histological investigation of the spleen showed a depression of hemopoiesis during the first 10 days but a return of hemopoietic function accompanied by the appearance of young blood cell forms occurs by the 30th day.

To elucidate the mechanism of such disturbances some experiments on the action of vibration and accelerations upon the mice hemopoietic organs were carried out. Evidence was found that mechanical factors are probably of significance in causing such changes. However, these results require further investigation. Author

[Translator's note: This is an original abstract and not a copy of the English "summary" printed with the article.]

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# EFFECTS OF COSMIC FLIGHT FACTORS ON THE INCIDENCE OF RECESSIVE LETHAL MUTATIONS IN THE X-CHROMOSOME OF DROSOPHILA MELANOGASTER

Ya. L. Glembotskiy, E. A. Abeleva, Yu. A. Lapkin, G. P. Parfenov

## I. Studies of Cosmic Radiation Effects on the Progeny of Drosophila

In 1925 the Soviet scientists G. A. Hadson and G. G. Philippov were the first to establish the mutagenic effect of ionizing radiations on yeast. In 1927 the American geneticist Muller published convincing evidence of this effect in the classical subject of genetic research, Drosophila.

This raised the question as to whether cosmic rays could also be the cause of spontaneous mutations. Theoretical calculations by Muller and Mott-Smith (1930), Efroimson (1931) and Timofeeff-Ressovsky (1931) based on the intensity of cosmic radiation on the Earth's surface and the frequency of mutations as a function of the radiation dose, suggested that cosmic radiation would not affect mutation rates. According to Efroimson's calculation (1931), cosmic rays could only be responsible for 1/140,000 spontaneous lethal mutations in Drosophila.

The Soviet scientist G. Frisen (1936) confirmed these calculations experimentally in 1935. The stratosphere balloon "USSR-I-a" which, on 26th June 1935, remained for two hours at 15,900 meters, contained about 3000 male Drosophila melanogaster flies of the wild Nal'chik strain. Using the standard CLB method on these males he determined the frequency of the recessive lethal mutations in the X-chromosome which is responsible for sex determination. These mutations are responsible for the death of the homozygote organism at an early stage in its development.

The earth's surface receives 1.4-1.5 ion pairs/$cm^3$/sec of cosmic radiation, while the stratosphere balloon "USSR-I-a" received 100-150 ion pairs/$cm^3$/sec. Hence their two hours' stay in the stratosphere these males received a radiation dose equivalent to 5.5-8.3 days on earth. Taking 20 days after hatching as a suitable age, the test and control males were then mated. The test males, after two hours in the stratosphere, had received a 28 to 42 percent greater radiation dose. If cosmic radiation played any appreciable part in spontaneous mutations, then the second generation of the test males should show a higher percentage of sex-linked recessive lethal mutations than the controls. In actual

fact, the mutations rates obtained were: progeny of stratosphere drosophila 0.294±0.104%; controls 0.409±0.129%.

Thus G. Frisen's tests confirmed the theoretical calculations which indicated that cosmic radiation had an insignificant effect on mutation rates. Geneticists then lost interest in the further study of this problem and for the next twenty years little work along Frisen's lines was carried out.

The historical launching of the first Soviet satellite on 4 October 1957, followed by others of increasing size containing better equipment, made cosmic flight by man a real possibility in the near future. It brought home the need for methods of protection against cosmic flight factors not only for the cosmonauts, but also for their progeny against genetic hazards. To guard against any danger, first of all we must be aware of its nature. Geneticists once more began to use Drosophila, which is a very suitable species for the study of the genetic effect of cosmic factors, since its high fertility, rapid sequence of generations and easily reproducible breeding conditions in the laboratory have enabled its genetics to be very thoroughly studied.

Sarah Pipkin and William Sullivan in the USA carried out the first tests using tubes containing Drosophila larvae which were carried in the USA Navy Scientific Research Stratosphere balloon (1959). The balloon rose to a height of over 18 km for 30.3 hours, including 16 hours at a height of 23.4 to 24.6 km. The Drosophila tubes were of glass and were kept at a temperature of 23.9° in a plastic woolen bag covered with a 5 cm thick layer of styrolene. This protected the larvae against sudden changes of temperature in the balloon gondola (which ranged from -1 to +35° C). The results of this cosmic radiation experiment were as follows. The larvae sent up to the stratosphere were the progeny of males characterized by four recessive factors: yellow body (gene y), white eyes (gene w), rough ocelli (gene ec) and undersized, twisted bristles (gene f), mated with normal (wild) females, of a grey body, red eyes of normal type and normal bristles.

The genes controlling these characters lie on the X-chromosome. In Drosophila the females have two X-chromosomes, one from the mother, the other from the father. Males have only one X-chromosome, from the mother. Hence all the female larvae which were in the stratosphere and subsequently developed to the imago were heterozygous, with four pairs of genes and genetic structure $\frac{++++}{y\ w\ ec\ f}$ in all body cells. While the male larvae and imago had a structure $\frac{++++}{\smile}$ (+ being the normal allelomorph

corresponding to the recessive gene, ♂ = y which is the one male chromosome). At first sight the progeny from such a mating would appear to be normal and not differ appreciably from the mother. They should have the same body, normal red eyes and normal bristles. If, however, in any body cell of a larva a mutation causing transition from the normal to the recessive allelomorph occurred, the imago body cells developing from similarly changed larva cells must differ from the surrounding cells developed from unaffected larval cells. Thus there would develop mosaic eyes, in which some of the ocelli would be white instead of red in color, or part of the body would be yellow instead of grey, etc. From the percentage incidence of such mosaics the frequency of such somatic mutations could be estimated.

Not one of the expected somatic mutations was observed among the 10,761 flies which had been in the stratosphere as larvae. A very small percentage of them showed other anomalies, e.g., a change in the shape and position of the wings, fewer bristles, absence of anal and vaginal plates, etc., but all such anomalies were also observed among the controls and at about the same frequency. No effect of the stratosphere flight on the viability and fertility of the flies was observed.

Evaluating the results of their tests, Pipkin and Sullivan showed that the irradiation dose to which the larvae were exposed did not exceed 20 mr. The smallness of the dose cannot be used to explain the absence of mutations. Lesions caused by the heavy particles of cosmic radiation must be considerably more serious than those caused by X- or $\gamma$-rays. Hence any evaluation of the mutagenic effect of cosmic radiation on the basis of the total irradiation dose must be very relative.

The impact of a heavy particle may trigger off some defect already present in the embryo or young larva. Many of the larvae in the stratosphere balloon at the time of the flight were still unhatched or had hatched not more than 7-1/2 h before the flight. Further, as the number of test and control eggs had not been counted, embryo mortality was not established and one of the probable effects of cosmic radiation could not be ascertained. It is also possible that with the low intensity of cosmic radiation at the height reached in the stratosphere and the comparatively small number of larvae per test tube the probability of heavy particles reaching the marked targets was low.

Pipkin and Sullivan therefore concluded that the height reached and the procedure applied were insufficient to determine the effect of cosmic radiation on the progeny of Drosophila. They considered it necessary to reach a height of at least 36 km and to apply more sensitive tests, in particular to use instead of larvae mature sperm, from mature males which are a more radiation sensitive test object.

## Tests During the Second Satellite Flight

The second satellite flight, at a height of about 320 km, lasted for 22 hours and offered incomparably better opportunities for investigating the effect of cosmic radiation on the progeny of Drosophila than stratosphere flights. However, apart from cosmic radiation, cosmic rocket flights involve other possible effects on the progeny, such as weightlessness, vibration during ascent and descent and, acceleration. At present we are still unable to differentiate between each of these factors and their interaction. On the basis of our results therefore, we can only consider the overall effect of all cosmic flight factors, and not of cosmic radiation alone.

Two tests were used to study the overall mutagenic effect of cosmic flight factors on Drosophila: 1) the frequency of sex-linked recessive lethal mutations (see above) on the X-chromosome; 2) the frequency of dominant lethal mutations (causing death at an early stage of development of the organism (a heterozygote according to this kind of mutations).

These tests were selected because lethal mutations, whether recessive or dominant, represent the greatest genetic risk for the progeny of cosmic space travelers; dominant for the first generation, recessive for later descendants. Moreover, these mutations are the most frequent of all.

According to Timofeeff-Ressovsky and Delbruck (1936), recessive lethal mutations in Drosophila number 20 percent and the dominant at least a further 20 percent of all spontaneous mutations. Among mutations induced by ionizing radiations the proportion of recessive and dominant lethals is still higher. Hence a study of the change of frequency of these two types of mutation gives some idea of the rate of change of the whole mutagenic process. The frequency of dominant lethal mutations is relatively simple and easy to determine by this test because the analysis applies entirely to the first generation of flies subjected to the mutagenic factor. As regards the determination of the frequency of recessive lethals, the method differs according to whether autosomes or sex chromosomes (X-chromosomes) are being studied. In autosomes the frequency of recessive lethal mutations is more difficult and slower to determine, because three generations of Drosophila are required, whereas two generations are sufficient for determining the frequency of recessive sex-linked lethals. Hence the test for recessive sex-linked lethal mutation is used most frequently for investigating the mutagenic process, and indeed most of our available knowledge is about sex-linked recessive lethal mutations induced by various mutagens.

These considerations induced us to select determination of the frequency of recessive sex-linked lethal and dominant lethal mutations as preliminary tests for the study of the mutagenic effect of a complex of cosmic flight factors.

A separate paper by P. G. Parfenov deals with the second of these tests. Only the results of the first test will be discussed here.

Test Procedure

The mutagenic effect of ionizing radiation differs according to the stage in the development of the sex cells which is irradiated. Numerous tests have shown that the frequency of mutations in mature sperm induced by X-rays, $\gamma$-rays and rapid neutrons is about 5-6 times greater than that occurring in spermatogonia; and in spermatids it is 1.5 to 2 times greater than in mature sperm (Glass, 1957; Glembotskiy, Abeleva, Lapkin, 1961). It has also been found that the effect of some chemical mutagens differs greatly in this respect from that produced by the kind of ionizing radiation referred to above. O. G. and M. I. Fahmy (1960) observed that nitro-mustard oil derivatives of amino acids, and in particular 2-phenylalanine, produced fewer sex-linked recessive lethals in mature sperms than in spermatogonia.

It would be of interest to know what the specific mutagenic effect of the complex of cosmic flight factors is in this connection. As a beginning to the solution to this problem it was decided to compare the mutability of two of the stages in spermatogenesis, the spermatid and mature sperm stages, both of which are characterized by maximum mutability when exposed to the types of ionizing radiation already investigated. It would be of equal interest to study the frequency of induced mutations as a function of the frequency of spontaneous mutations. There are Drosophila strains in which there is a considerable variation in their growth according to the frequency in their spontaneous mutations. The authors used two "wild" strains of Drosophila, in one of which (strain D-32) the mean recessive lethal mutations are 0.06 percent and in the other about 0.39 percent. How would these two strains, so different in the occurrence of their spontaneous mutations, respond to the mutagenic effect of different ionizing radiations? This question is both of theoretical and practical interest. Hence, as these strains were already being tested by the authors for the effects of $\gamma$-rays and rapid neutrons, they were also selected for use in the cosmic flight test.

About 400 males of each of two Drosophila strains were selected for the cosmic flight. Most of them had completed their pupal stage on the 15th August, i.e., were 4-5 days old at the time of the flight but some 25 percent of the males were 7 days older (pupal stage terminated 8th August). Males of equal age were placed together in the different flasks (see drawing, p. 254) and their progeny were also separately studied according to age. However, as the results for the progeny of both strains according to age proved somewhat complicated, they will be discussed together with those of all the test males irrespective of their age at the time of the flight.

On the morning of 22nd August the males were mated after their return from the second satellite flight. The Muller-5 method was used in the crossmating in order to establish the sex-linked recessive lethals. This is a well known method but it will be worth explaining it briefly so that less specialized readers can understand the test procedure and the results.

The "cosmic" males were crossed with females of the yellow Muller-5 strain. Externally the flies of the test strains D-32 and D-18 and of the yellow Muller-5 strain differ in that the two test strains have gray bodies and red eyes, while the yellow Muller-5 flies have a yellow body and yellowish (apricot color) eyes.

These phenotypical differences are due to the flies of the yellow Muller-5 strain having on their X-chromosome the two recessive genes - y (yellow body color) and $w^a$ (apricot eye color). The most important genotypic feature of this strain for the purpose of our test is the possession of X-chromosome inversion which prevents any crossing over, i.e., the interchange of homologous regions between two members of a pair of chromosomes during meiosis. This means that when yellow Muller-5 flies are mated with flies of another strain the X-chromosomes of both strains are passed on unchanged by the hybrids from one generation to the next (i.e., the chromosomes have the same structure as those the parents received from their respective parents).

The males of the first generation ($F_1$) produced by mating "cosmic" males with yellow Muller-5 females were of the gray red-eyed phenotype, but genotypically were heterozygotes $\frac{++}{yw^a}$ with two different X-chromosomes, one received from the "cosmic" father, $\underline{++}$, the other from the yellow Muller-5 mother. In a separate test each $F_1$ female was mated with a yellow Muller-5 male having one X-chromosome of type $\underline{yw^a}$. The second generation should produce: red-eyed gray females, genotype $\frac{++}{y\ w^a}$; apricot-eyed gray females of genotype $\frac{yw^a}{yw^a}$; gray, red-eyed $\underline{++}$ males; and yellow, apricot-eyed $\underline{yw^a}$ males. The X-chromosome $\underline{++}$ of the gray red-eyed males and females comes from the "cosmic" father. Any recessive lethal mutation of this chromosome will result in $F_2$ producing no gray males. In a similar $F_2$ breed the females will be of two phenotypes - red-eyed gray and apricot-eyed yellow, but there will be only one male phenotype -

apricot-eyed yellow. In order to establish definitely whether the absense of red-eyed gray males in the $F_2$ breed was due to a lethal mutation of the father's X-chromosome and not to some other accidental factor, all the gray red-eyed males of this breed were mated with yellow Muller-5 females. If the absence of red-eyed gray males was due to a lethal mutation then all the $F_2$ females of this breed would be heterozygotes and therefore will not produce red-eyed gray males. If the absence of such $F_2$ males was due to accidental causes, then red-eyed gray females of this breed will produce both apricot-eyed yellow and red-eyed gray males.

The percentage of red-eyed gray males absent in the $F_2$ mating indicates the frequency of the recessive lethal mutation in the X-chromosome of the "cosmic" males.

On the morning of 26th August the "cosmic" males were removed from the flasks where they had been kept for three days with yellow Muller-5 females and transferred for a further three days, until the morning of 29th August, to flasks containing new virgin yellow Muller-5 females, a mating which would produce progeny from sperm which at the time of the cosmic flight were only at the spermatid stage of development.

The frequency of recessive lethal mutations produced at this stage of spermatogenesis was analyzed by the method already described for testing the progeny produced from sperm which at the time of the cosmic flight were already mature.

The percentages of spontaneously occurring sex-linked recessive lethals occurring in the progeny of males of strain D-32 and D-18 from the first, second, seventh and eighth day after mating, were used as control. These figures had been obtained during the breeding of these strains under laboratory conditions in 1959 and 1960. The differences in the environment (apart from the complex of specific cosmic flight factors) of the "cosmic" males when compared with the controls consisted, firstly in their being kept on a "low diet" of only agar, sugar and yeast, without the usual raisins and semolina. The reason for this was that the greater consistency of the medium rendered it more resistant to shocks in the ascent and descent of the satellite. Secondly, for several days before the flight, when the flasks were already in the rocket, the ambient temperature for the flies reached 28-30$^{\circ}$, while under laboratory conditions the control males were kept at a temperature of 18-20$^{\circ}$ before mating.

Test Results and Their Evaluation

The table shows a significant statistically certain increase in the frequency of sex-linked recessive lethal mutations. The spermatid stage, in the progeny of the two strains, derived both from gametes which at the time of the cosmic flight were already mature and from gametes which then were at an earlier stage of development.

For strain D-32, characterized by a very low spontaneous rate of mutation, the mutation frequency increased 8 times, both for the sperms and spermatids. For strain D-18, with high spontaneous mutability, the mutation frequency increased 3 times for sperms and 10 times for spermatids.

The spermatids of strain D-32 produced more induced mutations than the mature sperm ($0.77\pm0.14\%$ and $0.44\pm0.08\%$). Strain D-18 gave similar results ($1.01\pm0.21\%$ and $0.78\pm0.23\%$). However, while the general tendency of both strains was towards a similar higher mutation frequency in the spermatids, in strain D-32 the ratio of mutation frequency of spermatids to sperms is 1.7, for strain D-18 it is only 1.3.

Similar results were, of course, obtained with these two strains also in mutation tests when they were subjected to small doses of $\gamma$-rays. As the gametogenesis time curve of strain D-18 may differ from that usual for Drosophila, some of the progeny resulting from mating 7-8 days after the mutagenic effect may have come from mature sperm or from spermatogonia and not from spermatids. At the present time this question is being further investigated by means of special tests. Until it is elucidated we can only say that assuming the usual period of spermatogenesis the mutagenic effect of the cosmic flight factor complex affects the spermatids of strain D-32 more than the mature sperm.

Although the relative increase in mutation frequency was greater in strain D-32 than in D-18, the latter showed a more pronounced absolute effect. According to the total data for both stages of gametogenesis the mutation frequency increased in the case of the low mutability strain D-32 by $0.56\pm0.07\%$, and for the high mutability strain D-18 by $0.86\pm0.16\%$.

Twenty of the lethal mutations in the progeny of "cosmic" males were subjected to a cytological analysis by examination of the salivary gland X-chromosomes. All these mutations proved to be point mutations, i.e., they showed no cytologically observable chromosome rearrangement.

Our data thus indicate that the cosmic flight had a considerable mutagenic effect on the progeny of Drosophila. The frequency of sex-linked lethals was considerably higher in the progeny of "cosmic" males than in that of the controls, and this effect was more pronounced in the progeny from gametes which at the time of the flight were in the spermatid stage of development. Strain D-18, characterized by a higher spontaneous

Frequency of Sex-linked Recessive Lethal Mutations in Drosophila Melanogaster, Due to a Complex of Cosmic Flight Factors

| Strain | Gametogenesis stage | Progeny of "cosmic" males | | | Progeny of control males | | | Mutation frequency induced by cosmic flight factors, %±m | Probability factor |
|---|---|---|---|---|---|---|---|---|---|
| | | Number of cultures | Lethals observed | | Number of cultures | Lethals observed | | | |
| | | | Number | %±m | | Number | %±m | | |
| D-32 | Sperm | 7,898 | 40 | 0.50±0.08 | 6,926 | 4 | 0.06±0.02 | 0.44±0.08 | 5.5 |
| | Spermatids | 4,755 | 42 | 0.88±0.13 | 5,505 | 6 | 0.11±0.04 | 0.77±0.14 | 5.5 |
| | Total | 12,653 | 82 | 0.64±0.07 | 12,431 | 10 | 0.08±0.02 | 0.56±0.07 | 8.0 |
| D-18 | Sperm | 2,150 | 25 | 1.16±0.20 | 8,243 | 31 | 0.38±0.03 | 0.78±0.23 | 3.3 |
| | Spermatids | 2,823 | 32 | 1.13±0.19 | 4,203 | 5 | 0.12±0.05 | 1.01±0.21 | 4.8 |
| | Total | 4,973 | 57 | 1.15±0.15 | 12,446 | 36 | 0.29±0.04 | 0.85±0.16 | 5.3 |

mutation rate than strain D-32, proved to be the more sensitive strain to the mutagenic effect of the complex of cosmic flight factors.

Which particular factors are responsible for the genetic effect produced in the experiment? Point mutations are characteristic of a mutation process induced by small doses of ionizing radiation. The more pronounced effect on spermatids than on sperms is also characteristic of ionizing radiation. In mature Drosophila sperm the effect of a dose of 1 r $\gamma$- or X-rays produces 0.003 percent(mean) sex-linked recessive lethals (Ives, 1959; Spencer, Stern, 1948). These figures refer to strains whose spontaneous mutability is in a ratio of 0.1-0.2 percent to that of the type of mutation tested. Of the strains used by us the D-32 approaches most closely to this ratio, and assuming that the mutagenic effect on the mature sperm of this strain (0.44 percent induced lethals) is due to ionizing radiation, it would correspond to their receiving a dose of 140-150 rad.

To what extent can we attribute this mutagenic effect on Drosophila to cosmic radiation? It has been shown that during cosmic flight living organisms are exposed not only to cosmic radiation but to other factors such as weightlessness, acceleration and rocket vibration. Possibly these specific cosmic flight factors may produce mutagenic effects by themselves or by interaction with cosmic radiation. It is known, for example, that the mutagenic effect of ionizing radiations such as X- or $\gamma$-rays may be considerably reinforced or weakened by the simultaneous effect of various chemical factors.

It should be borne in mind that the relative importance of these factors as mutagens (if proved to be such) will vary considerably according to the length and orbit of the cosmic flight.

The second satellite flight lasted for about 24 hours and its orbit did not pass through the earth's radiation layers. In orbit the satellite was not exposed to any sudden changes in the intensity of cosmic radiation. Under conditions of a relatively short flight not traversing regions of increased radiation, the mutagenic effect (if any) of rocket vibration and acceleration may play a considerable (possibly a decisive) part in the total mutagenic effect of the cosmic flight factor complex. On the other hand, the longer the flight, the more insignificant will be the effect of vibration or acceleration and the more important that of weightlessness and cosmic radiation, even of low dose intensities, in the total mutagenic effect.

To elucidate the nature of the total mutagenic effect of cosmic flight factors will require experiments in which the mutagenic effect of each separate factor and of their interaction are determined. Such a program should include: 1) the study of the effects of cosmic flights on the test subjects, e.g., Drosophila, in which some of the organisms were

protected from cosmic radiation and the rest were exposed to it; 2) the design of rockets in which the effect of weightlessness is eliminated; 3) the determination of the total mutagenic effect of rocket acceleration and vibration by short, vertical rocket flights in which the effect of weightlessness and cosmic radiation would be very slight; 4) the carrying out of terrestrial scale model tests in which special devices were used for the determination of the mutagenic effect of vibration and acceleration, both separately and jointly, and combined with ionizing radiation. Such tests have already begun.

## Conclusions

1. In two strains of Drosophila melanogaster (D-32 and D-18) a statistically significant increase in the frequency of sex-linked recessive lethal mutations was established which was caused by a complex of cosmic flight factors.

2. This mutagenic effect was found to occur in both the mature sperms and the spermatids of the two strains of Drosophila. It seems probable that the mutagenic effect on spermatids was more pronounced than on sperm.

3. Strain D-18, which is characterized by greater spontaneous mutability, proved to be more sensitive to the mutagenic effect of the whole complex of cosmic flight factors.

4. Twenty of the lethal mutations were cytologically analyzed and were shown to be point mutations.

5. The occurrence of point mutations and the increase in spermatid mutation rate, as compared with mature sperm, indicate the possibility of cosmic radiation being the responsible factor. But until the mutagenic effect of such cosmic flight factors as rocket vibration and acceleration has been elucidated by further experiments, the mutagenic effect of cosmic radiation alone cannot be accurately determined.

## Summary

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For two lines of Drosophila melanogaster a statistically reliable increase of mutation frequencies was observed after space flights (0.56 ± 0.07 percent and 0.96 ± 0.16 percent); the strain D-18, which is characterized by greater spontaneous mutability, proved to be more sensitive to the mutagenic effect of the whole complex of space-flight factors. Twenty of the lethal mutations were cytologically analyzed and were shown to be point mutations. The occurrence of point mutations and the increase in spermatid mutation rate, as compared with mature sperm,

indicate the possibility of cosmic radiation being the responsible factor. But until the mutagenic effect of such space-flight factors as rocket vibration and acceleration has been elucidated by further experiments, the mutagenic effect of cosmic radiation alone cannot be accurately determined.

Author

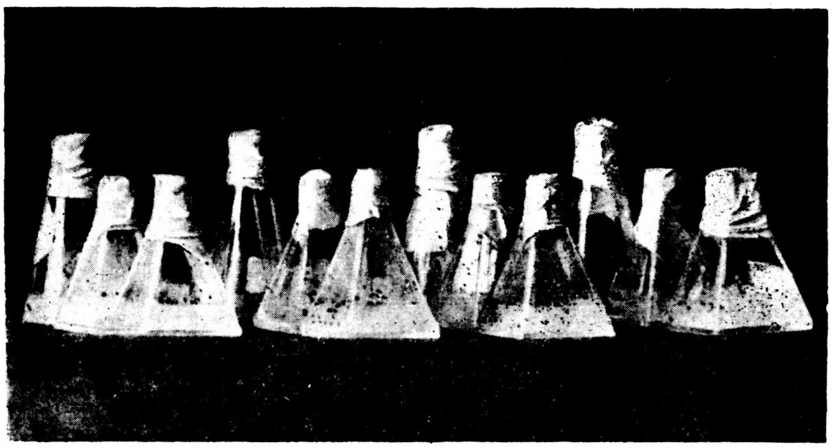

Figure 1. Flasks with Males of Drosophila melanogaster aboard a spaceship

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# INCIDENCE OF DOMINANT LETHAL MUTATIONS IN DROSOPHILA MELANOGASTER DURING A SATELLITE FLIGHT

G. P. Parfenov

Model experiments performed with some living organisms are of great value in assessing the danger to man of exposure to various mutagenic agents. The fruit fly, Drosophila, is specially suitable for such tests. The frequency of various types of mutations as a function of the radiation dose has been well established quantitatively and should be useful as a biological dosimeter in those cases where the physical analysis of mutagenic factors is difficult to estimate for any reason.

A number of investigators (Schaefer, 1950; Krebs, 1950) have shown that at great heights cosmic radiation may constitute a potential danger. However, the few tests which have been carried out in this connection have so far given negative results (Frizen, 1936; Pipkin, Sullivan, 1959). This may possibly be due to the fact that at the heights reached insufficient exposure of the test subjects occurred. The second satellite contained Drosophila flies in flasks. The progeny of these flies was analyzed to determine the frequency of dominant lethal mutations. The test is comparatively simple as mutation results can be detected in the first generation of flies.

## Methods

Some 800 males of the wild type, strain D-32, 4-5 days old at the time of the flight, were used. On August 22nd these males were placed in a vessel (diameter 150 mm, height 300 mm) together with the same number of virgin females of the same strain. After six hours slides, on which was a film of agar-agar (30 g/l water), were placed in the vessel. The females laid eggs on the slides and after 4 hours the number of eggs laid was counted and fresh slides were placed in the vessel. After two days the slides were reexamined to determine the number of eggs which did not develop. On August 23rd, 24th and 25th eggs were selected for the determination of dominant lethal mutations in the male spermatozoa. On the evening of August 25th females of the yellow Muller-5 strain were placed in the vessel for fertilization by the males and the collection of eggs was discontinued.

On August 29th all females were removed and replaced by new virgin females of strain D-32. On August 29th and 30th eggs were collected for determination of the dominant lethal mutations in the male gametes which at the time of the cosmic flight were in the spermatid stage.

There were no dominant lethal mutations among the controls. All undeveloped eggs were considered as unfertilized. The percentage of induced dominant mutations was determined by the difference between the number of undeveloped eggs in the test and control groups.

## Results and Discussion

The flight in the satellite (as can be seen from Table 1) produced no increase in the frequency of dominant lethal mutations in gametes which were in the mature sperm stage at the time of the flight. However, gametes in the spermatid stage showed a small but statistically significant increase in the number of mutations (2.56±0.63). As the spermatid stage is specifically radiosensitive (Glass, 1957) it is possible that this increase may be due to the action of cosmic radiation. This effect could, however, be due also to other cosmic flight factors, such as vibration, acceleration, weightlessness or a combination of all these.

Table 1. Frequency of Dominant Lethal Mutations in Drosophila Melanogaster, Strain D-32, Due to Cosmic Flight Factors

| Stage of gametogenesis | Group | Number of eggs laid | Number of undeveloped eggs | Percentage of undeveloped eggs | Frequency of mutations induced by cosmic flight factors | Probability factor |
|---|---|---|---|---|---|---|
| Sperms | Controls | 3,106 | 241 | 7.76±0.48 | 0.60±0.95 | 0.63 |
| | Test | 1,136 | 95 | 8.36±0.82 | | |
| Spermatids | Controls | 6,571 | 389 | 5.92±0.29 | 2.56±0.63 | 4.06 |
| | Test | 2,511 | 213 | 8.48±0.55 | | |
| Total | Controls | 9,677 | 630 | 6.51±0.25 | 1.93±0.52 | 3.71 |
| | Test | 3,647 | 308 | 8.44±0.46 | | |

Table 2 shows the frequency of dominant lethals caused by vibrations of 70 c/s and 0.4 mm amplitude. The flies were subjected to the vibrations twice for 15 min, at an interval of 24 hours, using the facilities available to us. Comparison of the data in Tables 1 and 2 shows that vibration produced the same number of dominant lethals in spermatids as occurred during the cosmic flight. Moreover, it shows also a significant increase in the number of dominant lethals in sperm. It would, however,

Table 2. Frequency of Dominant Lethal Mutations in Drosophila Melanogaster, Strain D-32, Due to Vibration

| Stage of gameto-genesis | Group | Number of eggs laid | Number of undeveloped eggs | Percentage of undeveloped eggs | Frequency of mutations induced by vibrations | Probability factor |
|---|---|---|---|---|---|---|
| Sperms | Controls | 7,415 | 533 | 7.19±0.30 | 2.17±0.48 | 4.52 |
| | Test | 5,771 | 540 | 9.36±0.38 | | |
| Spermatids | Controls | 5,082 | 449 | 8.83±0.40 | 2.55±0.69 | 3.69 |
| | Test | 3,259 | 371 | 11.38±0.55 | | |
| Total | Controls | 12,497 | 982 | 7.81±0.08 | 2.28±0.40 | 5.70 |
| | Test | 9,030 | 911 | 10.09±0.32 | | |

be premature to assume that the dominant lethals caused by the flight were due solely to rocket vibration because in the ascent the flies are exposed to a wide vibration spectrum and in our laboratory tests low frequency vibrations were used. Lastly, the undeveloped eggs in both tests need not necessarily be attributable to chromosome aberrations because the number of such eggs may increase owing to temporary impotence of the test males. Only the first steps have so far been taken in the study of dominant lethal mutations in Drosophila melanogaster caused by space flights. Further detailed analysis of the basic factors involved in cosmic flights is necessary. It would be premature to draw any practical conclusions from the material so far available.

Summary

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After flight in a space satellite the frequency of dominant lethal mutations which occurred in spermatids of male Drosophila, strain D-32, was significantly increased. No increase in these mutations was observed in mature spermatozoa. However, the frequency of dominant lethal mutations was also increased in both spermatids and spermatozoa when male flies were subjected to vibrations of 70 c/s on Earth. No firm conclusions can be drawn, at present, on the contribution of vibrational effects to the induction of mutations during space flight.

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# INFLUENCE OF SPACE-FLIGHT FACTORS ON HEREDITY AND DEVELOPMENT IN ACTINOMYCETES AND HIGHER-ORDER PLANTS

1st al

Ya. L. Glembotskiy, A. A. Prokof'yeva-Belgovskaya,
Z. B. Shamina, V. V. Khvostova, S. A. Valeva, et als
N. S. Eyges and L. V. Nevzgodina

Problems of Space Genetics

Many investigations by Soviet and foreign scientists have established that X-rays, $\gamma$-rays and fast neutrons are powerful agents which can cause changes in the bodies of animals. The effect of radiation on the nuclei of sex cells and on their basic structural elements (chromosomes) is to bring about a variety of changes (mutations) in the organisms which develop from these germinal cells. In most cases these changes are harmful. Soviet scientists N. P. Dubinin, M. A. Arsen'yeva and Yu. N. Kerkis (1960) have established that radiation doses of the order of 10 r cause considerable changes in the chromosomes of sex cells of rodents and monkeys, whereby the number of chromosome changes in monkeys is twice as high.

Experiments carried out by N. P. Dubinin, Yu. N. Kerkis and L. I. Lebedev (1960) in which human tissue was irradiated with X-rays have shown that a dose of the order of 10 r doubles the mutation rate. It is known that the biological effectiveness of various types of ionization radiation differs. For instance, fast neutrons are known to induce 1.5-2 times, and in some cases dozens of times, as many mutations as X- or $\gamma$-rays.

The first experimental investigations on the mutagenic effect of cosmic rays were carried out in 1935 by the Soviet scientist G. G. Frizen (1936). In 1959 a similar experiment by the American investigators S. B. Pipkin and W. N. Sullivan was carried out in which Drosophila were flown in the atmosphere. These experiments were carried out in stratostats which rose for a short period to altitudes not exceeding 23 to 25 km. Neither of these investigations revealed any genetic effect.

The Soviet satellite carried mice, fruit, Drosophila flies, spiderwort (Tradescantia), seeds of wheat, pea, corn (maize), spring onion and Nigella as well as samples of actinomycetes (producers of antibiotics) and other biological material.

At present, we can only discuss the genetic effects induced by the entire set of space-flight factors. One of the tasks of subsequent

investigations will be to elucidate the separate role of the individual factors and of their interrelations.

## Influence of Space-Flight Factors on the Growth and Development of Actinomycetes

Three strains were used in the experiments: Actinomycetes erythreus strains 2577 and 8594 and Actinomycetes streptomycin Kras strain LS-3. The strains were obtained from the Selection Division of the All-Union Scientific Research Institute of Antibiotics.

The cultures were grown on agarized corn (Act. erythreus) and pea media (Act. streptomycin). They were stored in a refrigerator (2 to 6°C) before the experiment. During the experiment the control cultures remained in the refrigerator.

After the space flight the control and experimental cultures were investigated in the following way:

1. Viability was determined by inoculating Petri dishes with spores and counting the number of colonies that developed.

2. The microscopic characteristics of the cultures growing on a liquid maize medium in a rocker at 27 to 28° C were determined.

The strains 2577 and 8594 differ in the dimensions of the nuclear apparatus of their spores and their sensitivity to the effects of ultraviolet radiation. The spores of the strain 2577 contain a large nuclei and are resistant to the effects of ultraviolet radiation. In the strain 8594 the nuclei in the spore are smaller and are less sensitive to ultraviolet radiation. It can also be assumed that they differ with respect to their reaction to ionizing radiation.

## Experimental Results

### Viability of the Strains Actinomycetes Erythreus

Data on the viability of the strains 2577 and 8594 are given in Table 1.

Both strains reacted differently to spaceflight conditions. In strain 2577, which has large nuclear elements and appears to be a highly polyenergid system (resistant to ultraviolet radiation), the space flight increased the viability of the spore germination and the number of developing colonies to about six times that of the controls. In strain 8594,

which is sensitive to ultraviolet radiation and has smaller nuclei, spore viability under space-flight conditions decreased sharply.

Table 1. Influence of the Experimental Conditions on the Viability of Two Actinomycetes Erythreus Strains

| Group | 2577 | | 8594 | |
|---|---|---|---|---|
| | No. of colonies (per $cm^3$ of spore suspension) | % | No. of colonies (per $cm^3$ of spore suspension) | % |
| Control | 1,290 | 100 | 25,000 | 100 |
| Experimental | 8,600 | 666 | 2,000 | 8.3 |

Microscopic Analysis of the Growth and Development of Actinomycetes Erythreus and Actinomycetes Streptomycin Kras Strains

The experimental and control cultures of all the three strains were subjected to two passes (sub-inoculations) in well oxygenated liquid culture media. For cytological analysis of the culture, specimens of the culture liquid were taken during the entire period of growth (5 days). Preparations of mycelium were fixed in Carnoy fluid and stained with methylene blue and giemsa stains according to Robino.

The results showed that spaceflight conditions stimulated growth in all the three strains. Both during the first and second pass (sub-inoculation) the intensity of growth was higher than for the controls. This is indicated by the following features of the cytology of the experimental cultures:

a) The development of stronger (thicker) and more basophilic hyphae (Figure 1) Act. erythreus 2577, first pass (sub-inoculation);

b) the growth of a denser web of mycelium (Figure 2) Act. erythreus 8594;

c) prolonged growth of well-developed hyphae: during the fifth day of incubation the hyphae of the control culture of the strain Act. streptomycin LS-3 either formed spores, or those which did not form spores were autolyzed, i.e., the culture terminated its life cycle. The mycelium of the experimental culture, however, was still in a state of full maturity, forming a dense mass of well-developed, strong (thick) hyphae (Figure 3).

Thus, the experimental conditions stimulated intense mycelial growth not only in the strains which were resistant to ultraviolet radiation but

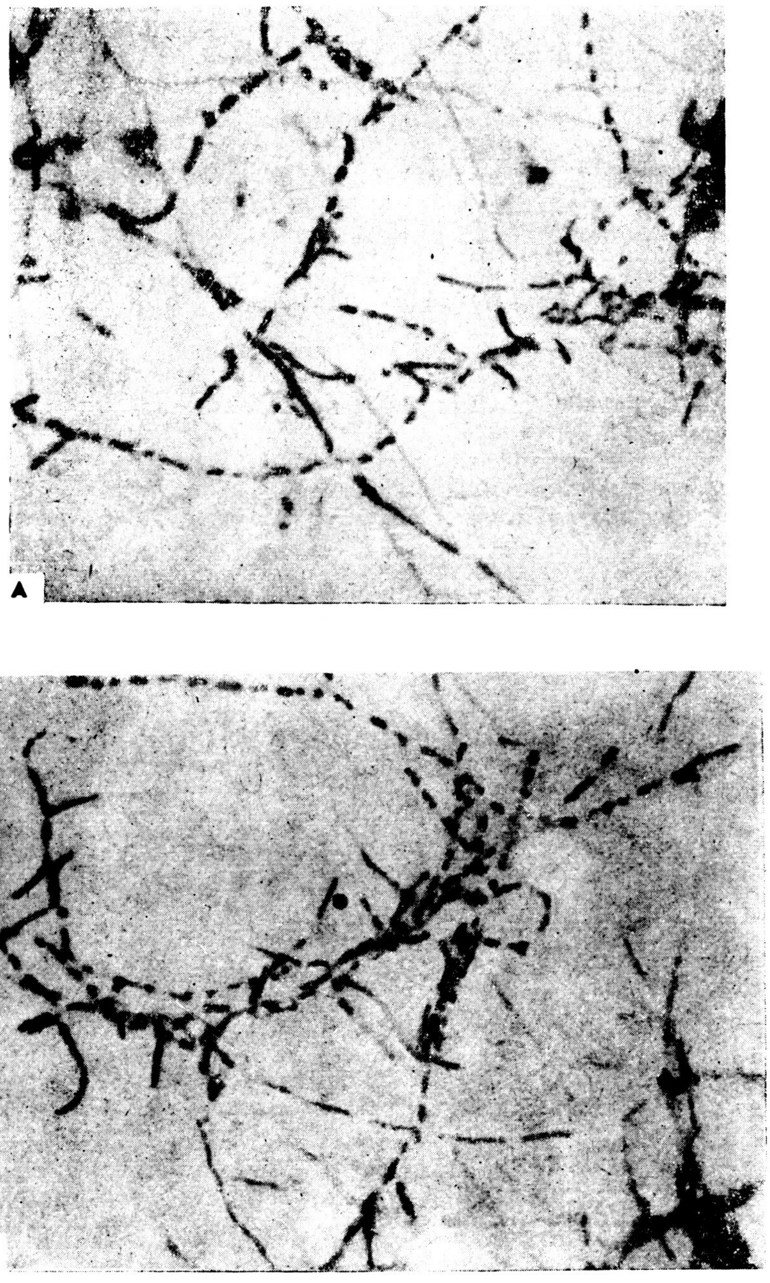

Figure 1. Actinomycetes Erythreus, Strain 2577, First Pass (Sub-inoculation), Deep Culture, X2000

A - Experimental B - Control

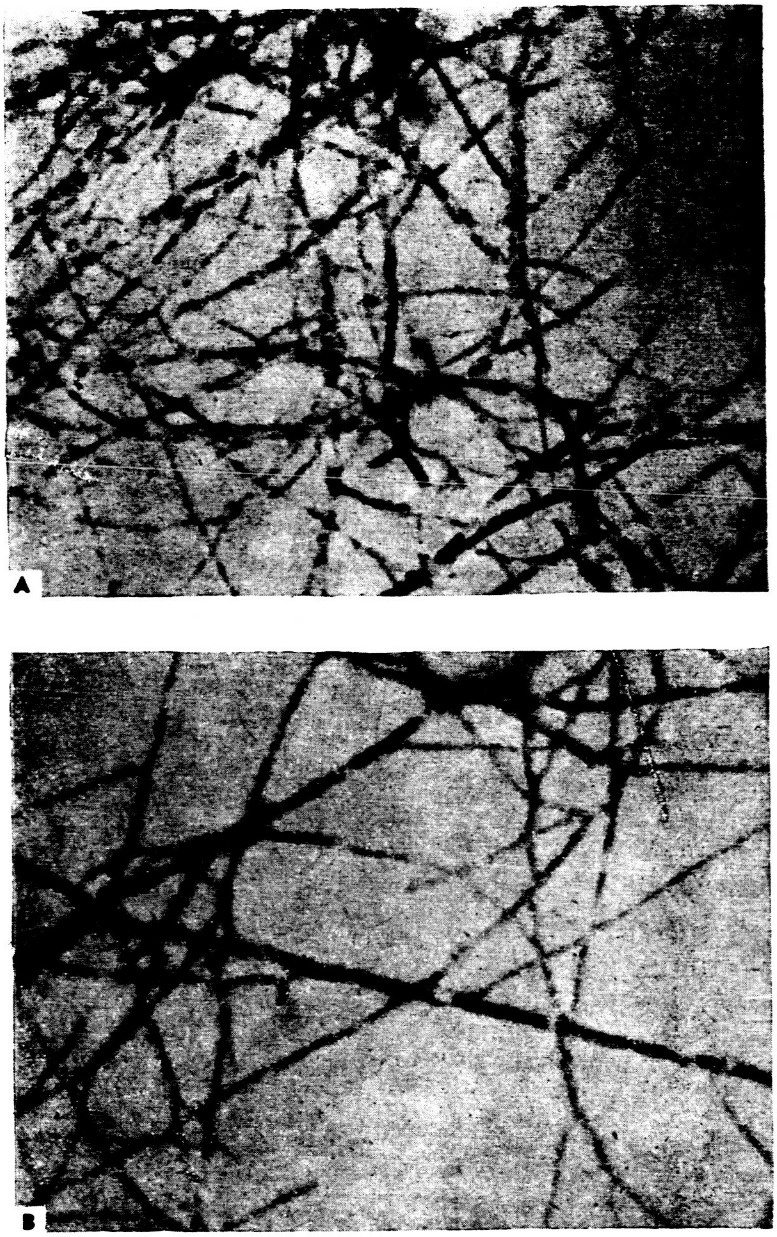

Figure 2. Actinomycetes Erythreus, Strain 8594, Second Day of Growth, Second Pass (Sub-inoculation), X2000

A - Experimental B - Control

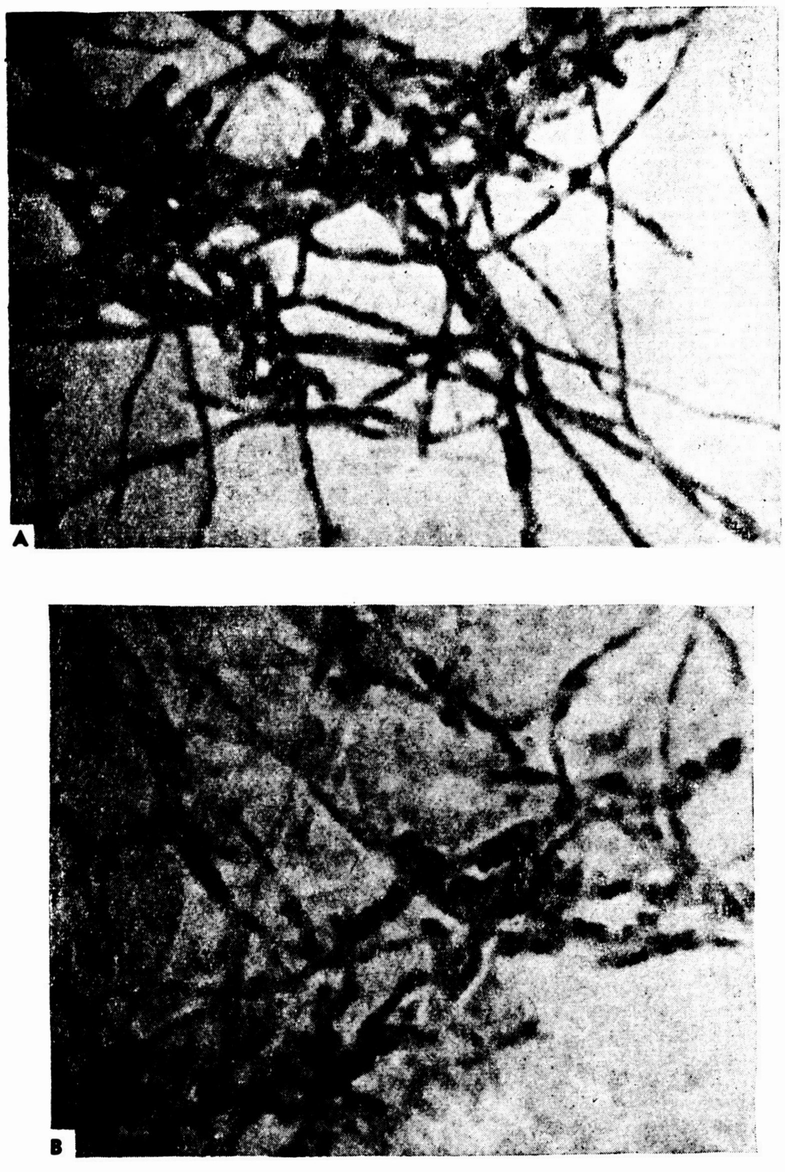


Figure 3. Actinomycetes Streptomycin Kras, Strain LS-3, Fifth Day of Growth, Second Pass (Sub-inoculation), X2000

A - Experimental B - Control

also in those spores of the radiation-sensitive strains 8594 which retained their viability.

Viability of the Culture of Actinomycetes Aureofaciens Strain LSB-2201

The data obtained on the stimulation of mycelial growth of the three strains of Actinomycete in liquid culture are reasonably reliable. However, data on the survival rate of the strains 8594 and 2577 are not sufficiently reliable, since the concentrations of the spore suspension of the controls and of the experimental cultures were determined visually from the cloudiness of the suspension. Due to their minute dimensions (0.5 to 1.0$\mu$) it was not possible to count the number of the actinomycete spores.

Therefore, in the next experiment (fourth Soviet satellite) the culture used was Act. aureofaciens, strain LSB-2201, which was kept on millet grains. The density of the suspension of the spores of the control culture and of the culture after the experiment was determined spectrophotometrically. The optical density D of the aqueous suspensions of the spores of Act. aureofaciens, as a function of wavelength, is given below:

| Wavelength, Å | Experimental | Control |
|---|---|---|
| 3500 | 0.132 | 0.130 |
| 3690 | 0.222 | 0.211 |
| 3700 | 0.113 | 0.110 |
| 3800 | 0.100 | 0.098 |

It can be seen that the values of the optical density (which depends on the number of spores in the suspension) in the experimental and control cultures were very similar.

After inoculation on an agar medium and incubated for 10 days at 28° C, the number of growing colonies was counted. The average number of colonies per 1 ml of suspension amounted to 13132 in the experimental and 55417 in the control; the percentage of survival in the experimental cultures relative to the control cultures was 24 percent.

The fifth satellite also carried the same culture LSB-2201. Preparations of spore suspensions from the experimental and control cultures were filtered through cotton wool and filter paper. The "experimental" suspension was transparent, while the "control" suspension remained cloudy. This cloudiness was caused by the millet in the media on which

the culture was maintained. The "control" suspension was, therefore, strongly diluted in distilled water and then filtered; even so the cloudiness remained. It was not possible to determine the density of this suspension by the spectrophotometric method. However, it can be reliably stated that in spite of being cloudy, the "control" suspension was much more fluid, i.e., it contained less spores than the "experimental" suspension.

After inoculating the media with spores the number of colonies that developed was counted and the average number of colonies per 1 ml of suspension determined. It amounted to 9033 in the experimental suspension and 34166 in the control. The percentage of survival in the experimental cultures relative to the control was 26.4 percent.

It can be seen that the viability of cultures of Act. aureofaciens LSB-2201, which were subjected to the effects of space flight on the fourth Soviet satellite was 24 percent; and on the fifth satellite, the viability of similar cultures relative to the control was 26.4 percent. In other words, the spaceflight factors lowered the viability of cultures of this variety of Actinomycete by an average of 75 percent.

However, even when the spectrophotometric method is used it is very difficult to obtain absolutely identical "control" and "experimental" spore suspensions. Therefore, the data on Actinomycetes given about should be considered as being only of a preliminary nature.

## Morphologic Mutability

The morphological mutability of the colonies of Act. erythreus 8594 and Act. aureofaciens LSB-2201 (in percent) was also investigated:

| Culture | Experimental | Control |
|---|---|---|
| Act. erythreus 8594 | 0.23 | 0.31 |
| Act. aureofaciens LSB-2201 | 0.35 | 0.41 |

These data indicate that the morphological mutability of Actinomycetes from the experimental (flown in space) cultures are within the same limits as the control cultures.

## Cytological Analysis of the Influence of Space-Flight Factors on Seeds of Agricultural Plants

Air-dried seeds of two types of pea were used - (1) Spartanets which is resistant to $\gamma$-radiation and (2) Maslichnyy which is sensitive to $\gamma$-radiation. Two types of maize - (1) Nemchinovskaya (resistant to

γ-radiation and fast neutrons) and (2) Podmoskovnaya (sensitive to these types of radiation) and (3) a winter wheat - couch grass hybrid 186 (sensitive to γ-radiation and fast neutrons) were also tested.

The seeds used in the experiment and the control seeds were sown simultaneously in Petri dishes on filter paper. After growing to a length of 8 to 12 mm in the case of the peas and maize, and 4 to 6 mm in the case of the wheat, the radicles were fixed in a mixture of 96-deg alcohol and glacial acetic acid (at ratios of 3:1) for 4 hours. Then they were transferred into 70-deg alcohol where they were stored pending further investigation. The first cell divisions (mitosis) were investigated by squashing the root tips and staining them with aceto-carmine. Germination begins with cell growth and mitosis, which occurs in the different plants at varying times. Therefore, the germinating roots were allowed to grow to a certain length before they were fixed. Shorter shoots of wheat were fixed since mitosis first occurs in these earlier than in peas and maize (corn). An analysis is carried out in which a mitotic index is determined of the frequency of cell divisions. The total number of cells and the number of dividing cells were counted in 10 fields of every preparation. In each preparation the mitotic index was calculated for 500 cells and the variation over series was worked out, from which the average mitotic index was calculated.

The injury to the chromosomes was studied in the ana- and telophases of the first mitosis. The number of cells was counted with "bridges" and fragments which form as a result of the chromosomes breaking up. The uninjured chromosomes migrate to the poles in the anaphase stage and are dragged away (from the metaphase equatorial plate) by the fibers of the spindle. These protein fibers adhere to the centromere of the chromosomes. If a section of the chromosome breaks and has no "centromere" it will not migrate to the poles. Such an acentric fragment can be easily detected in the cytoplasm between the chromosomes which migrate to the poles. If two chromosomes are broken and an interchange of fragments occurs, chromosomes with two centromeres may be formed. Centromeres in the anaphase frequently migrate to different poles and dicentric chromosomes form a bridge in the cell (Figure 4). During the breaking-up of chromosomes, numerous other rearrangements may occur but the method applied by the authors only permits taking into consideration certain types of chromosome abnormalities which serve as indicators of the frequency chromosomal rearrangement, the total number of which is really much larger.

The obtained results (Table 2) indicate that in all those specimens studied which were exposed to space-flightconditions a certain increase in the percentage of cells with chromosome rearrangement was observed. But only in two specimens (winter wheat and Spartanets pea) was this increase statistically reliable.

Table 2. Percentage of Anaphases (A) and Telophases (T) with Chromosome Rearrangement and Mitotic Index of Sprout Rootlets

| Object | Group | No. of shoots investigated | No. of A+T rearrangements | No. of analyzed A+T | A+T with rearrangements, % | Difference between experimental and control | Index of reliability of difference | Average mitotic index | Difference between mitotic indices of experimental and control | Index of reliability of differences in mitotic indices |
|---|---|---|---|---|---|---|---|---|---|---|
| Pea Spartanets | Control | 10 | 6 | 857 | 0.35±0.2 | 1.29±0.42 | 3 | 7.6±0.35 | 3.3±0.57 | 5.8 |
| | Experimental | 10 | 18 | 1098 | 1.64±0.37 | | | 10.9±0.45 | | |
| Pea Maslichnyy | Control | 12 | 12 | 1505 | 0.39±0.14 | 0.37±0.24 | 1.5 | 8.75±0.43 | 1.7±0.59 | 1.82 |
| | Experimental | 12 | 18 | 1831 | 0.76±0.2 | | | 9.82±0.42 | | |
| Maize (corn) Nemchinovskaya | Control | 10 | 3 | 1013 | 0.29±0.17 | 0.33±0.41 | 0.75 | 7.8±0.4 | 0.6±0.7* | 0.86 |
| | Experimental | 16 | 10 | 1601 | 0.62±0.38 | | | 7.2±0.6 | | |
| Maize (corn) Podmoskovnaya | Control | 10 | 7 | 1028 | 0.68±0.25 | 0.70±0.41 | 1.7 | 4.6±0.3 | 2.9±0.36 | 8 |
| | Experimental | 13 | 17 | 1232 | 1.38±0.33 | | | 7.5±0.2 | | |
| Wheat PPG-186 | Control | 33 | 26 | 1654 | 1.84±0.33 | 4.96±0.68 | 7.3 | 8.06±0.17 | 1.47±0.25* | 5.7 |

* In the experiment the method index is lower.

Figure 4. Normal Anaphase, Left; Anaphase with Chromosome Bridge and a Dual Fragment, Right

It is interesting that in three specimens (both types of pea, Podmoskovnaya corn) there was such an increase in the mitotic index (rate of cell division) that in two of the specimens this was statistically reliable. In the corn - type Nemchinovskaya - the rate of cell division did not change, while in the wheat it slowed down somewhat.

These differences are regular: The type PPG-186 has the highest number of chromosomes, 42; pea, 14 and corn, 20. It is understandable, therefore, that its percentage of chromosomal rearrangement (and in the "control") is higher than in the other specimens, and increases in the "experimental" material by 5 percent. The constancy of cells with chromosomal rearrangement in the Nemchinovskaya-grade corn is also understandable since it is resistant to $\gamma$-radiation and to neutrons.

The reaction of the pea Spartanets was unexpected. In this specimen the increase in the number of cells with chromosomal rearrangement was statistically reliable. It is known that this type is resistant to $\gamma$-radiation, but its reaction to fast neutrons has not yet been investigated.

In seeds where the percentage of ana- and telophases with chromosomal rearrangement exceeded unity (pea Spartanets, corn Podmoskovnaya) the increase in the mitotic index was also statistically reliable. The increase in the fraction of anaphases with chromosomal rearrangement to 5 percent (wheat) resulted in a decrease in the mitotic rate.

## Conclusions

1. All the four strains of Actinomycetes (Act. erythreus strain 8594 and 2577, Act. streptomycin strain LS-3 and Act. aureofaciens strain LSB-2201) proved sensitive to space-flight conditions.

2. In the radiation-resistant strain 2577, its viability (number of surviving spores and developed colonies) increased sixfold as compared with the control; in the radiation-sensitive strain 8594 it decreased twelvefold.

3. In shoots of all the five kinds of experimental seeds there was an increase in the percentage of chromosome rearrangement, but only in two varieties was this increase statistically reliable.

4. In three varieties of plant an increase in the rate of cell division was observed. When the percentage of anaphases with chromosome rearrangement was high (about 5 percent) the rate of cell division decreased.

## Summary

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The following four Actinomycete strains were tested: Act. erythreus, strains 8594 and 2577, Actinomyces streptomycini Kras., strain LS-3, and Act. aureofaciens, strain LS-B-2201. All these proved sensitive to the effects of spaceflight factors. Actinomyces erythreus, strain 2577, had a sixfold increase in viability (measured by the number of spores which survived and colonies which developed) as compared with the controls. The viability of Actinomyces erythreus 8594, a radiosensitive strain, was reduced to one-twelfth, and that of Actinomyces aureofaciens LS-B-2201 to one quarter. Actinomyces streptomycini Kras. strain LS-3 formed spores, while its mycelium autolyzed.

Air-dry seeds of two types each of corn and peas and one of winter wheat were exposed to space flight, following which chromosome damage in the root cells of the seedlings was investigated. Both types of peas and one of corn showed increases in the mitotic index. However, only in the Podmoskovskaya corn type and "Spartanets" pea type was the mitotic index statistically significant. All of the seeds showed chromosome rearrangements. Winter wheat (hybrid 186) showed the greatest number, which was to be expected since this hybrid is sensitive to both $\gamma$-rays and fast neutrons. However, the "Spartanets" pea type, which is resistant to $\gamma$-rays, also showed a statistically significant number of chromosome rearrangements.

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# INFLUENCE OF SPACE-FLIGHT CONDITIONS ON SEEDS OF ALLIUM FISTULOSUM (SPRING ONION) AND NIGELLA DAMASCENA (NUTMEG FLOWER)

V. N. Sidorov and N. N. Sokolov

The effect of cosmic radiation on the hereditary structure of the body under conditions of space flight is of major interest. For this reason, we selected as objects of investigation dry seeds of two types of plants which differed very greatly in radiation sensitivity: Allium fistulosum, highly sensitive to radiation, and Nigella damascena, resistant to radiation. Some data characterizing the X-ray sensitivity of seeds of these species as assessed by chromosomal rearrangement are given in Table 1.

Table 1. Frequency of Chromosome Rearrangements in Dry Seeds of Allium Fistulosum and Nigella Damascena Irradiated with a Dose of 2500 r

| Specimen | Group | No. of examined cells | Cells with aberrations | |
|---|---|---|---|---|
| | | | No. | % |
| Allium fistulosum | Control | 1611 | 16 | 0.99±0.24 |
| | Irradiated with 2500 r | 2478 | 456 | 18.40±0.78 |
| Nigella damascena | Control | 1204 | 3 | 0.25±0.14 |
| | Irradiated with 2500 r | 1541 | 29 | 1.88±0.35 |

It can be seen from this table that the sensitivity of Allium is more than nine times as high as that of Nigella. The choice of specimens with so greatly differing sensitivities to radiation enabled accurate characteristics of the effect of cosmic radiation to be obtained for both large and small effects.

Comparison of the number of aberrations which occurred in seeds that were flown in the second satellite with the corresponding control, which were germinated at the same time, revealed a complete absence of any influence of space-flight conditions on the frequency of chromosome rearrangement in seeds of both species studied (Table 2).

Table 2. Influence of the Conditions of Space Flight on the Frequency of Chromosome Rearrangements in Allium Fistulosum and Nigella Damascena

| Specimen | Group | No. of examined cells | Cells with aberrations | |
|---|---|---|---|---|
| | | | No. | % |
| Allium fistulosum | Control | 1377 | 42 | 3.05±0.46 |
| | Experimental | 1910 | 54 | 2.83±0.37 |
| Nigella damascena | Control | 822 | 2 | 0.24±0.17 |
| | Experimental | 983 | 3 | 0.31±0.17 |

Because negative data were obtained when dry seeds were used, it was considered necessary to use in subsequent experiments, plants in more sensitive stages of development, for instance, moistened, germinated seeds, the radiation sensitivity of which is usually tens of times higher than that of dry seeds.

More interesting data were obtained from germinating experimental and control seeds. It was found that space-flight conditions undoubtedly had a stimulating effect on the sprouting of seeds of both species. Data on the rate of sprouting of the seeds are given in Tables 3 and 4 and plotted in Figures 1 and 2.

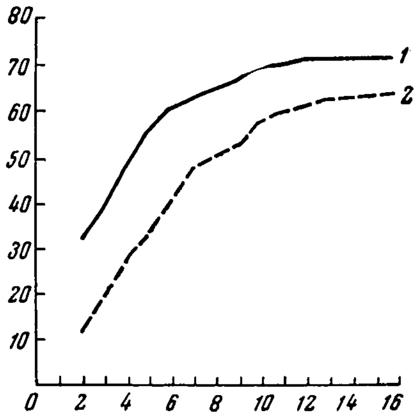


Figure 1. Rate of Germination of Allium Fistulosum (Spring Onion) Seeds. Ordinate, Percentage of Sprouting Seeds, Along the Abscissa, Time of Germination, in Days.

Curve 1 - Seeds Which Have Returned from Space Flight
Curve 2 - Control Seeds

Table 3. Rate of Germination of Seeds of Allium Fistulosum with Time (Days) in Percent (n = 286)

| Days | 2 | 3 | 4 | 5 | 6 | 7 | 9 | 10 | 11 | 12 | 13 | 14 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | 12.2 | 19.5 | 28.3 | 33.0 | 41.6 | 47.2 | 53.1 | 57.7 | 60.8 | 61.5 | 62.3 | 63.6 | 64.0 |
| Experimental | 32.5 | 39.5 | 48.2 | 55.0 | 60.5 | 63.3 | 66.7 | 69.7 | 70.0 | 71.0 | 71.0 | 71.0 | 71.0 |

Table 4. Rate of Germination of Seeds of Nigella Damascena with Time (Days) in Percent (n = 295)

| Days | | 4 | 5 | 6 | 7 | 9 | 10 | 11 | 12 | 13 |
|---|---|---|---|---|---|---|---|---|---|---|
| Control | | | | | | | 0.3 | 0.3 | 1.7 | 1.7 |
| Experimental | | 13.6 | 19.7 | 26.8 | 42.0 | 53.9 | 56.3 | 57.6 | 58.7 | 58.7 |
| Days | 14 | 16 | 17 | 18 | 19 | 20 | 21 | 23 | 24 | 25 |
| Control | 3.4 | 6.1 | 8.8 | 11.6 | 13.2 | 14.6 | 16.6 | 20.3 | 22.0 | 22.4 |
| Experimental | 59.3 | 59.7 | 61.0 | | | | | | | |

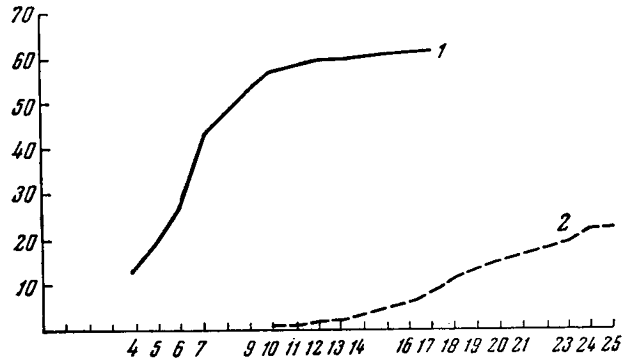


Figure 2. Rate of Germination of Nigella Damascena (Nutmeg Flower) Seeds. Ordinate, Percentage of Sprouting Seeds, Along the Abscissa, Time of Germination, in Days

Curve 1 - Seeds Which Have Returned from Space Flight
Curve 2 - Control Seeds

The data indicate that conditions of space flight cause acceleration in the rate of germination of seeds as well as an increase in the ability to germinate. The stimulating effect is particularly pronounced in the species which is less radiation-sensitive, namely, the Nigella damascena. This fact indicates that the observed stimulating effect of the flight conditions is not due to radiation since, in this case, the effect should have been detected in the species which has a higher radiation sensitivity.

This conclusion follows from the existence of a known relation between the radiation sensitivity and the stimulating dose. It is known that the higher the radiation sensitivity, the smaller is the radiation dose required for producing a stimulating effect.

Obviously, the stimulation is likely to be due to some other factors (Translator's note: for instance, weightlessness, acceleration, etc.) which act during space flights.


Summary

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The effect of space flight on seeds of Allium fistulosum and Nigella damascena was studied. There was no influence on the frequency of chromosome rearrangements, but the rate of seed germination was markedly increased in Nigella damascena but not in Allium fistulosum. This latter effect does not appear to be due to exposure of the seeds to cosmic radiation, but may depend on other factors encountered in flight.

Author

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# SPACE-FLIGHT FACTORS AND PRIMARY NONDISJUNCTION OF CHROMOSOMES

N. P. Dubinin and O. L. Kanavets

The main categories of mutations which occur either naturally or are induced mutation processes produced artificially, are gene mutations, structural chromosome rearrangements, mutations of genomes causing polyploidy, and nondisjunction of chromosomes which lead to the appearance of hyperploids and aneuploids. The phenomenon of nondisjunction of chromosomes is due to the fact that two homologous chromosomes or chromatids go to the same division pole during anaphase. This phenomenon has been studied in numerous plant and animal species. Muntzing (1961) has made a classical analysis of primary and secondary nondisjunction in Drosophila, thorn apple and wheat.

In recent years, it has been found that nondisjunction of chromosomes in humans leads to the birth of children with serious hereditary diseases. It was established (Davidenkov and Efroimson, 1961; Efroimson, 1961; Efroimson, 1960) that such serious diseases as Klinefelter's syndrome, Turner's syndrome, Down's syndrome and others can be caused by the presence of additional chromosomes in the Karyotype of the diseased person. These extra chromosomes get into the egg cell as a result of nondisjunction of the sex chromosomes or of the autosomes. If nondisjunction of the X-chromosomes occurs during oogenesis, abnormal egg cells will develop containing either two X-chromosomes or no X-chromosomes at all. When these are fertilized with normal sperm, half of which possess X- and the other half Y-chromosomes, abnormal zygotes containing XXY, XXX, YO and XO will appear. XXX individuals have a low viability and YO zygotes are nonviable. Thus, nondisjunction of sex chromosomes leads to the formation of XXY and XO individuals who are characterized by a series of anomalies. XXY individuals are men displaying Klinefelter's syndrome. Individuals with the genetic structure XO are women who suffer from Turner's syndrome. The general picture of sex heredity in both Man and Drosophila and the results of primary nondisjunction of sex chromosomes are presented in Sketch 1. In it the difference in the mechanism of chromosomal determination of sex in Man and Drosophila is taken into consideration. This difference is associated with the active role of the Y-chromosome in determining the male sex in Man while in Drosophila the Y-chromosome plays basically the role of a partner in the disjunction of the sex chromosomes.

It is known that the nondisjunction of chromosomes may be due to several factors and, in the first instance, it was shown that it could be caused by ionizing radiation (Mavor, 1924; Lobashev, 1937; Tsivin, 1956; Tikhomirova, 1961).

In view of the important biological consequences of nondisjunction of chromosomes, we consider that the investigation of the influence of space-flight factors on the processes of primary nondisjunction is of major interest. A homozygous, white-eyed strain of Drosophila was chosen for testing. Unfertilized females of this strain were used as a "biological indicator" in the spaceship "Vostok I", in which the first Soviet astronaut, Yu. A. Gagarin, was placed in orbit on April 12, 1961. After landing on April 15, 1961, the white females were mated with red-eyed males. From April 15 to April 29, 1961, these females were transferred to new cultures every three days. The virgins were selected on March 28, 29 and 30 so that on the day of the flight, April 12, 1961, they were two weeks old. The results of analysis of the process of nondisjunction of the sex chromosomes of Drosophila are given in Table 1, where data are summarized characterizing the first generation produced by the females which were subjected to space-flight conditions.

The number of abnormal individuals (white-eyed females and red-eyed males) in the offspring of females that had been subjected to space-flight conditions and were due to primary nondisjunction of the X-chromosomes amounted to 0.19%±0.021.

The abnormal individuals were subjected to supplementary genetic analysis. The females were investigated for secondary nondisjunction; the males for fertility. All the abnormal males did indeed have the genetic constitution XO, which was proved by their sterility. Fifteen white-eyed females had 796 offspring, of which 71, i.e., 8.9 percent, were abnormal. The number of abnormal offspring which resulted from secondary nondisjunction varied, depending on the genotype of the strain. The number of abnormal offspring which were produced indicates that the abnormal females which appeared in the progeny of the flies that were subjected to space-flight conditions did indeed have the genetic structure XXY.

White-eyed females of the same strain, bred in a similar manner, were used as controls. They were also brought to the launching pad and were subjected to the same effects as the experimental ones, with the exception of the space flight itself. The data characterizing the first generation of this group of control females are given in Table 2. The number of abnormal individuals (white-eyed females and red-eyed males) which arose as a result of the primary disjunction of the X-chromosomes in the progeny of the control females that had not been subjected to space-flight conditions was 0.048±0.0017.

Thus, the number of cases of primary nondisjunction of X-chromosomes in Drosophila females increases as a result of the conditions of space flight. Under these conditions, the percentage of nondisjunction was four times as great as that in the controls.

Table 1. Influence of Space-Flight Conditions on the Process of Nondisjunction of Sex Chromosomes

| Age of females, days | Females | | | Males | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|
| | Normal | Ab-normal | Abnormal, % | Normal | Ab-normal | Abnormal, % | Normal | Ab-normal | Abnormal, % |
| 16-18 | 4,535 | 2 | 0.044±0.030 | 3,956 | 10 | 0.253±0.079 | 8,491 | 12 | 0.141±0.040 |
| 19-22 | 6,349 | 9 | 0.141±0.047 | 5,413 | 21 | 0.388±0.084 | 11,762 | 30 | 0.255±0.047 |
| 23-26 | 6,023 | 6 | 0.100±0.040 | 4,680 | 8 | 0.173±0.061 | 10,703 | 14 | 0.133±0.036 |
| 27-29 | 3,319 | 6 | 0.081±0.073 | 3,129 | 8 | 0.257±0.090 | 6,448 | 14 | 0.218±0.058 |
| 30-32 | 2,980 | 7 | 0.235±0.089 | 2,734 | 7 | 0.257±0.096 | 5,714 | 14 | 0.244±0.065 |
| Total | 23,206 | 30 | 0.129±0.022 | 19,912 | 54 | 0.271±0.039 | 43,118 | 84 | 0.195±0.021 |

Table 2. Nondisjunction of Sex Chromosomes of Control Individuals Not Subjected to Space-Flight Effects

| Age of females, days | Females | | | Males | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|
| | Normal | Ab-normal | Abnormal, % | Normal | Ab-normal | Abnormal, % | Normal | Ab-normal | Abnormal, % |
| 20-24 | 2,396 | 1 | 0.042±0.041 | 2,133 | 1 | 0.047±0.047 | 4,529 | 2 | 0.044±0.031 |
| 25-27 | 2,796 | 0 | 0 | 2,575 | 3 | 0.116±0.067 | 5,371 | 3 | 0.057±0.033 |
| 28-29 | 1,484 | 1 | 0.067±0.067 | 1,413 | 0 | 0 | 2,897 | 1 | 0.035±0.035 |
| 30-34 | 1,278 | 1 | 0.078±0.078 | 1,221 | 1 | 0.083±0.083 | 2,499 | 2 | 0.080±0.056 |
| 35-37 | 604 | 0 | 0 | 625 | 0 | 0 | 733 | 0 | 0 |
| Total | 8,855 | 3 | 0.034±0.017 | 7,967 | 5 | 0.063±0.028 | 16,826 | 8 | 0.048±0.017 |

What is the cause of the increase in the frequency of primary nondisjunction of chromosomes under the influence of space-flight conditions As far as the influence of cosmic radiation is concerned, the dose which they received during the flight of the spacecraft "Vostok 1" on April 12, 1961, was very small indeed. Cosmic radiation during this flight could hardly be the cause of an increase in the primary nondisjunction of chromosomes. Mavor (1924) found that after X-irradiation the increase in the primary nondisjunction was only observed for 6 days after exposure. In our case, the incidence of primary nondisjunction was increased on the average over the entire 18 days during which the females were tested (see Table 1).

This indicates that qualitatively the disruption of the processes of mitosis which lead to nondisjunction of chromosomes is more extensive and lasts longer in the case of space flight than in the case of exposure to X-rays. Further investigations will be required to elucidate the causes of these changes.

It is known (Plough, 1917) that in Drosophila females the development of germ cells from oogonia to mature eggs takes six days. Thus, X-rays act on the cells during maturation. Space-flight factors influence not only this stage but also the oogonia. However, in both cases, the processes of nondisjunction are associated with disturbances in the cell divisions leading to mature eggs. The fact that the causes of nondisjunction are due to disturbances in the processes of cell division indicates that they may be caused by vibration of the satellite on takeoff and landing, the state of weightlessness during flight, or a complex combination of factors.

## Conclusions

It was found that the conditions of space flight increased the incidence of the primary nondisjunction of X-chromosomes in Drosophila females. This effect is prolonged and differs from the known, relatively short-duration after-effects which arise in the case of X-ray radiation; the cause of the increased incidence of primary nondisjunction, inasmuch as they act on the mechanism of cell division, is the state of weightlessness during flight, vibration of the satellite or a more complex combination of factors.

The investigations carried out earlier in the Laboratory for Radiation Genetics of the Institute of Biophysics, Academy of Sciences, USSR, established that in Drosophila the conditions of space flight bring out an increase in the frequency of appearance of recessive, lethal and dominant mutations and also crossing over (Glembotskiy et al, 1961; Parfenov, 1961). In this paper information is given on the discovery of a new class of genetic after-effects caused by space flights which are of great importance for the offspring of Man and that of all organisms that

Sketch 1. Inheritance of Sex in Man and Drosophila and Results of Primary Nondisjunction of Sex X-chromosomes

| Normal male gametes | Normal female gametes | Exceptional female gametes | |
|---|---|---|---|
| | X | XX | 0 |
| X | XX[1] | XXX[3] | XO[5] |
| Y | XY[2] | XXY[4] | YO[6] |

1. Female, in both Man and Drosophila.
2. Male, in both Man and Drosophila.
3. Zygote of female sex and of low viability in Drosophila (superfemale).
4. In Man - male with Klinefelter's syndrome; in Drosophila - fully viable female.
5. In Man - female with Turner's syndrome; in Drosophila - sterile male.
6. Zygote nonviable.

accompany him on space flights. This problem has to be analyzed in greater detail. Furthermore, it is necessary to take into consideration that, the progeny injured by primary nondisjunction of chromosomes represent a very small percentage, when expressed in absolute numbers. However, this may be of appreciable importance for microorganisms which reproduce very quickly and which may produce several generations during a flight. In the first instance, this factor will have to be taken into consideration from the point of view of populations of such organisms.

Summary

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In order to test the effects of space flight on the nondisjunction of chromosomes, homozygotic, white-eyed, unfertilized female Drosophila were used. Upon return, they were exposed to red-eyed males. The percentage of offspring affected by space-flight factors was 0.19% ± 0.021. When white-eyed control females were bred in a similar manner the percentage of spores caused by nonseparation of the X-chromosome was only 0.048% ± 0.017. The percentage of nonseparation in those exposed to space flight was four times greater than in the controls.

The amount of radiation absorbed during the flight of Vostok I was too small to account for this change. In addition, laboratory experiments with X-rays have indicated that radiation effects are of short duration, lasting only six days. However, flies exposed to space-flight factors

produced an equally high percentage of mutants during the entire 18-day study, indicating an equally high number of chromosome separation failures. This indicates that space-flight factors, unlike radiation, affect not only oocytes and maturing ova but also the oogonia. Therefore, it seems probable that the factors responsible for the disruption of chromosome separation are due to vibration, weightlessness, or combined factors.

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# INFLUENCE OF SPACE-FLIGHT FACTORS ON THE LEVEL OF SEROTONIN IN THE BLOOD OF ANIMALS

V. S. Shashkov, V. V. Antipov, M. O. Raushenbakh,
G. A. Chernov, and V. A. Maslennikova

The number of factors which act on living organisms during space flights requires extensive study of the general biological, physiological-biochemical and other changes which may occur during and after flight.

In the complex process of adaptation to the unfavorable effects of the external environment, changes in hormones and enzymes play an important role, and may reflect both the action and the mobilization of the compensatory-adaptation control mechanisms (Orbeli, 1935; Speranskiy, 1935; Selye, 1952; Ogle, 1957). Ogle (1957) assumed that various steroids, hormones, and other biochemical substances may serve as valuable indicators for determining the state of the functions of the body and its individual systems, as well as its resistance to unfavorable effects.

The importance of the pituitary and suprarenal glands in the stress reactions and the process of adaptation has been conclusively proved by the work of G. Selye and his school (1960). In his work the role of such hormones as adrenaline and noradrenaline is emphasized.

Following the discovery of serotonin (5-Hydroxytryptamine), much attention has been paid during the last decade to this biogenous amine. An extensive world literature has accumulated, including a number of fundamental review papers which are devoted to the physiological significance of serotonin, its role in the control of the functions of the central and peripheral nervous systems, and its effect on permeability; the tone of unstriated muscle structure, as well as the metabolism and biosynthesis of this substance (Erspamer, 1953; Page, 1954, 1958; Chernov, 1958, 1960; Mogilevskiy, 1960).

The established close relation of serotonin with noradrenaline, dopa-amine and acetylcholine, on the one hand, and of these amines with corticosteroids and macroergic (high energy content) phosphates, on the other hand, is of interest (Sourkes, 1958; Holtz, Baltzer and Westermann, 1957; Ramey and Goldstein; 1959; Richard and Davis, 1957).

With reference to radiation pathology, many authors (Gray et al., 1952; Langendorff et al., 1957; Zherebchenko et al., 1960) have shown the pronounced protective action of serotonin against radiation in various types of animals. This is of particular importance, since the basic danger which threatens man in space is associated with cosmic radiation.

Taking into consideration the importance attached to serotonin in the control of physiological and biochemical functions and of its role as a radio-protective agent, we have studied the content of the amine in the blood of the animals flown into space in the fourth and fifth Soviet orbital spaceships, at various times after landing.

The content of serotonin in the blood was determined by a biological method previously described by several authors (Erspamer, 1953; Vane, 1957). The method is based on the ability of serotonin to cause contraction of the smooth muscle fibers of various organs, in the given case the musculature of an isolated section of the intestines of a rat.

The laboratory animals carried in the satellites were placed in cages, the bottom of which was lined in one case with "paralone", which reduces the effects of vibration, and in another case with perspex.

Two groups of animals were used as controls. Of these, one group was put through all the conditions of the experiment except for the flight and was returned to the laboratory, together with the animals which had returned from the satellite. The second control group consisted of animals which were kept throughout under ordinary living conditions.

The changes in the serotonin level in the blood of the mice and dogs are given in Tables 1 and 2.

It can be seen from the data listed in the tables that the serotonin concentration in the blood of the control animals was 0.12-0.2 μg/ml. The content of serotonin in the blood of the mice which were dispatched to and returned from the place of launching hardly differed from that of the control animals. The amine content of the mice who had been in space decreased 8 to 10 times one - two days after the flight. Later on, the serotonin concentration returned to the level of the controls.

The serotonin content in the blood of the experimental dogs who flew in the satellites are given in Table 2, from which it can be seen that, in the same way as in the mice, a drop in the serotonin level (3.5-10 times) could be observed during the first and second days after flight and later the levels returned to normal values.

Examination of the blood of the dogs (Belka, Strelka, Shutka, Zhul'ka, Kusachka, Kometa, Pestraya) carried out a considerable time - from the 80th to the 240th day - after the space flight did not show any deviations in the serotonin level of the blood from the normal (the amine concentration was 0.12-0.28 μg/ml).

Thus, the results of the investigations indicate that after a space flight the serotonin level decreases, then returns to normal within a relatively short period, and remains at that level.

Table 1. Serotonin Content in the Blood of Mice at Various Periods after Return from Flight

| Satellite | Date of investigation | Time after flight, days | Group | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Control I (mice in animal house) | | Control II (mice transported to launching pad) | | Mice that flew in cage with paralone bottom | | Mice that flew in cage with perspex bottom | |
| | | | No. of animals | Serotonin concentration, $\mu g/ml$ | No. of animals | Serotonin concentration, $\mu g/ml$ | No. of animals | Serotonin concentration, $\mu g/ml$ | No. of animals | Serotonin concentration, $\mu g/ml$ |
| Fourth | 10 Mar 61 | 1st | 3 | 0.18 | 3 | 0.098 | - | - | 3 | 0.058 |
| | 11 Mar 61 | 2nd | 3 | 0.18 | 3 | 0.15 | 2 | 0.089 | 2 | 0.086 |
| | 20 Mar 61 | 10th | 2 | 0.138 | 2 | 0.2 | 2 | 0.13 | 2 | 0.135 |
| | 9 Apr 61 | 30th | 2 | 0.128 | 2 | 0.19 | 2 | 0.14 | 2 | 0.14 |
| Fifth | 27 Mar 61 | 2nd | 2 | 0.2 | 2 | 0.2 | 4 | 0.063 | 2 | 0.012 |
| | 4 Apr 61 | 10th | 2 | 0.14 | 2 | 0.15 | 2 | 0.12 | 2 | 0.1 |
| | 24 Apr 61 | 30th | 2 | 0.2 | 2 | 0.18 | 2 | 0.14 | 2 | 0.16 |
| | 24 May 61 | 60th | 2 | 0.135 | 2 | 0.12 | 2 | - | 2 | 0.14 |

Since during flight on rockets and satellites the body is subjected to a complicated series of effects, including acceleration, vibration and radiation, it is of interest to study the effect of these factors separately. In this case the effect of vibration on serotonin content has been studied.

Table 2. Serotonin Content in the Blood of Dogs at Various Times after Space Flight

| Time after flight, days | Fourth satellite Dog Chernushka | | Fifth satellite Dog Zvezdochka | |
|---|---|---|---|---|
| | Investigation date | Serotonin concentration, μg/ml | Investigation date | Serotonin concentration, μg/ml |
| Before flight | 24 Feb 61 | 0.135 | 8 Mar 61 | 0.128 |
| 1st | 10 Mar 61 | 0.037 | - | - |
| 2nd | 11 Mar 61 | 0.39 | 27 Mar 61 | 0.012 |
| 5th | 14 Mar 61 | 0.1 | 30 Mar 61 | 0.126 |
| 10th | 19 Mar 61 | 0.125 | 4 Apr 61 | 0.156 |
| 20th | 29 Mar 61 | 0.127 | 14 Apr 61 | 0.18 |
| 30th | 8 Apr 61 | 0.13 | 24 Apr 61 | 0.19 |
| 40th | 18 Apr 61 | 0.136 | 8 May 61 | 0.2 |
| 60th | 8 May 61 | 0.128 | 28 May 61 | 0.23 |

These investigations were made on mice and guinea-pigs under terrestrial conditions. The mice were subjected to vibration with a frequency of 35 and 70 cps, the guinea-pigs to vibration of 70 cps for 15 min with a total overload of 10 g and an amplitude of 0.4 mm. The animals were killed at various times after the vibration tests.

The results of these investigations are summarized in Table 3 and it can be seen that immediately after the vibration the serotonin level in the blood of the mice and the guinea-pigs decreased by a factor of 4.3 - 10. In mice which were subjected to vibration of a frequency of 70 cps, a decrease in the serotonin content was observed up to 48 hours after the vibration but in the case of 35 cps vibration, the serotonin level returned to normal (0.1-3 μg/ml) within the first day.

In guinea-pigs the concentration of serotonin returned to the values of the controls during the second day.

Comparing changes in the serotonin level in the blood of animals after space flights and in the blood of animals subjected to vibration, it can be seen that they are identical, both in direction and in the magnitude of the decrease. It should also be noted that the time taken for the

Table 3. Concentration of Serotonin in the Blood of Animals at Various Times after Vibration

| Time of killing after vibration | Mice | | | | | | | | Guinea pigs | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 70 cps | | | | 35 cps | | | | 70 cps | | | |
| | Test animal | | Control | | Test animal | | Control | | Test animal | | Control | |
| | No. of animals | Serotonin content, μg/ml | No. of animals | Serotonin content, μg/ml | No. of animals | Serotonin content, μg/ml | No. of animals | Serotonin content, μg/ml | No. of animals | Serotonin content, μg/ml | No. of animals | Serotonin content, μg/ml |
| 15 minutes | 6 | 0.01 | 3 | 0.13 | 3 | 0.02 | 3 | 0.13 | 2 | 0.046 | 2 | 0.2 |
| 60 minutes | 6 | 0.013 | - | - | 3 | 0.047 | - | - | - | - | - | - |
| 120 minutes | 6 | 0.019 | - | - | 3 | 0.058 | - | - | 2 | 0.042 | 2 | 0.19 |
| 240 minutes | 6 | 0.012 | 3 | 0.15 | 3 | 0.037 | 3 | 0.13 | - | - | - | - |
| 24 hours | 6 | 0.08 | 3 | 0.14 | 3 | 0.11 | 3 | 0.2 | 2 | 0.089 | 2 | 0.22 |
| 48 hours | 6 | 0.011 | 3 | 0.135 | 3 | 0.1 | 3 | 0.19 | 2 | 0.21 | 2 | 0.24 |
| 10 days | 6 | 0.12 | 3 | 0.129 | 3 | 0.12 | 3 | 0.13 | 2 | 0.21 | 2 | 0.24 |
| 30 days | 6 | 0.15 | 3 | 0.22 | 3 | 0.13 | 3 | 0.12 | 2 | 0.23 | 2 | 0.21 |
| 60 days | 6 | 0.13 | 3 | 0.18 | 3 | 0.1 | - | 0.12 | - | - | - | - |

serotonin content to return to normal was the same in both cases. This justifies making an association between the changes in the serotonin level of animals under space-flight conditions with those under the action of vibration.

It is known that the serotonin content of the blood is almost entirely concentrated in the platelets (Bracco, Curti and Ballerini, 1954; Weissbach et al., 1958), although data are available which indicate that serotonin may also be adsorbed by other elements in the blood Translator's note: (Form elements are defined as the substances which are suspended in the plasma, i.e., primarily the red and white blood cells and the thrombocytes.)(Hardisty and Stacey, 1955; McIsaac and Page, 1959). It has also been proved that serotonin is not formed in the thrombocytes themselves since these do not contain 5-hydroxytryptophan decarboxylase, an enzyme which decarboxylates 5-hydroxytryptophan - the product from which the amine is synthetized.

Serotonin is synthetized in enterochromaffin cells of the mucous membrane of the gastro-intestinal tract (Idem, 1954, 1957; Dalgliesh, Toh and Work, 1953) from where it enters the blood stream.

There are indications that it may be formed in the central nervous system and in other tissues containing 5-hydrooxytryptophan decarboxylase (Bogdanski, Weissbach and Udenfriend, 1956).

It is assumed that in the body serotonin is mainly present in a combined (pharmacologically inactive) state. The free amine is rapidly deaminated by monoaminoxydase. It is possible that acetylation of serotonin and formation of esters with glucuronic acid and sulfate may occur (McIsaac and Page, 1959; Idem, 1957; Blaschko, 1952; Sjoerdsma, Smith et al., 1955).

The information available at present on the formation and metabolism of serotonin does not provide data from which the mechanism of the lowering of its concentration in the blood of animals subjected to space flight conditions over long periods or to the effects of vibration can be elucidated. However, it is not associated with changes in the number of the platelets since their level remained normal in the peripheral blood of both groups of animals. The mechanism of decrease in the serotonin content of the blood of the animals under space-flight factors requires further study.

SUMMARY

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Changes in the serotonin (5-oxytriptamine) level in the blood of animals subjected to flight on orbital spaceships IV and V have been studied by Vane's method (ability of serotonin to cause contraction of

smooth muscle fibers). Control animals kept at the home laboratory and those taken to the cosmodrome had identical (0.12 to 0.2 μg/ml) serotonin concentrations. Dogs flown in the spaceships showed an eight to tenfold drop in the serotonin level one to two days after flight. After two days this level returned to normal and remained normal up to 240 days.

Similar reductions (by a factor of 4.3 to 10) in this level were obtained in mice and guinea pigs subjected to vibrations (35 and 70 cps, amplitude 0.4 mm) for 15 minutes accompanied by acceleration overloads of 10 g. Restoration to normal levels of serotonin in the blood was noted after two days in the case of mice subjected to vibrations of 70 cps and after one day in the case of those subjected to 35 cps.

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# DETERMINATION OF THE INDUCED RADIOACTIVITY IN THE SECOND SOVIET ARTIFICIAL SATELLITE

V. V. Matveyev and A. D. Sokolov

While in orbit and passing through the radiation belts, the structural materials of the satellite were subjected to continuous bombardment with cosmic rays. Nuclear reactions in the structural materials which accompany this cosmic-ray bombardment may produce radioactive nuclei.

Accumulation of induced radioactivity may create additional sources of radioactive radiation in long duration space flights which will result in an increase of the level of the ionizing radiation to which the plants and animals on board will be subjected.

Due to the absence of the necessary data on the composition of cosmic radiation and its effect on various structural materials, it is not possible to estimate theoretically the induced radioactivity. Experimental determination of the radioactivity induced in the various materials of the satellite or spaceship is of definite scientific and practical interest.

In the here described work an attempt has been made to determine the residual gamma-activity in the material of the "bioelement" located on board the second Soviet satellite [Translator's note: Bioelements (AMN-1, Bios) - Instruments specially developed for automatic recording of the biological activities of microorganisms. Bioelements AMN - transmit to the ground information on the life functions of bacteria and permit biological characterization of the conditions of existence of living cells in zones of space difficult to penetrate with larger animals.]

To obtain the maximum possible information on phenomena taking place in the investigated material under the effect of hard cosmic radiation, it was considered best to analyze the induced radioactivity by means of (full absorption) $4\pi$-scintillation spectrometers. Such spectrometers have a high recording efficiency for $\gamma$-radiation (practically 100 percent for energies up to 500-600 kev), they permit determining not only the absolute activity of preparations with very low radioactivity but also judging the isotope composition from the $\gamma$-radiation energy spectra.

The bioelements investigated were of special design in the form of a cylinder 38 mm diameter, 40 mm high and weighing about 86 g. Since the authors did not have available spectrometric crystals with such large cavities and it was impossible to ensure $4\pi$-geometry recording by other

methods, it was decided to utilize for the measurements a type GSD-4π sensor (Arsayev, Makarov, Mamikonyan, Matveyev, 1950).

The sensor consists of two spectrometric scintillation counters (sodium iodide crystal of 120 mm diameter, 70 m high [Translator's note: surely mm not m], with a type FEU-24 photoamplifier), which are provided with a 55 mm thick lead shield, and an electronic circuit for simultaneous recording of the signals from both counters and summing of their amplitudes.

From the sensors the pulses were fed to the input of a single-channel amplitude analyzer type ASS-1 (Gorn, Ivanov, Khazanov, 1960), at the input [Translator's note: Output?] of which standard counters were connected.

Selection of the optimum regime for feeding the photomultipliers, minimum level of discrimination, as well as determination of the linearity of the amplitude characteristics and the resolving power of the galvanometer were all carried out by means of conventional techniques using suitable radioactive isotopes.

The radioactivity of both the bioelement which returned to Earth from the second Soviet satellite and the control bioelement which remained on the ground was measured. During the measurements, the bioelement was provided with a shield and was placed on a special support between the end faces of the scintillation counter crystals. The spectrometer operated under conditions of integral counting.

The investigations have shown that the residual gamma-activity of the bioelements, 15 days after return to Earth, does not exceed $10^{-10}$ g-eq radium, if it exists at all.

SUMMARY

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The possibility of the materials and instruments of a satellite becoming radioactive during flight in space cannot be ruled out. The authors determined the residual γ-activity of bioelements AMN-1 (instruments specially developed for automatic recording of the biological activity of microorganisms under space conditions), used in the second Soviet satellite. The measurements were made by means of a 4π-scintillation (absorption) counter 15 days after the return to Earth of the capsule. At that time the residual γ-activity, if present at all, did not exceed $10^{-10}$ radium gram equivalent.

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# SOME RESULTS OF MEDICAL AND BIOLOGICAL INVESTIGATIONS IN THE SECOND AND THIRD SATELLITES

V. V. Antipov, R. M. Bayevskiy, O. G. Gazenko, A. M. Genin, A. A. Gyurdzhian, N. N. Zhukov-Verezhnikov, B. A. Zhuravlev, L. I. Karpova, G. P. Parfenov, A. D. Seryapin
E. Ya. Shepelev and V. I. Yazdovskiy

## Investigations on the Second Satellite

One of the important problems in ensuring life support for animals during flight in satellites is to produce and maintain a gaseous medium, the composition, pressure and temperature of which will be such that the basic physiological functions are not inhibited.

Dogs and other biological material can withstand relatively large fluctuations in the ambient medium; this applies to the content of oxygen and carbon dioxide in the exhaled air as well as to the pressure and temperature. For instance, dogs can withstand a reduction in air pressure to 460 mm Hg for long periods, which corresponds to an altitude of 4000 m above sea-level or a reduction in the oxygen content of the inhaled air to 15-13 percent. The concentration of carbon dioxide can increase from 0.03 to 4 percent without causing any appreciable metabolic disturbances in animals.

However, when preparing a life-support system it was essential to create the most favorable conditions for the animals. For this reason it was necessary to keep the permissible fluctuations of the parameters of the air in the capsule within very narrow limits. This is important because any appreciable deviations in the conditions of the medium from the normal limits will impose additional stresses to a greater or lesser extent which, in turn, will put additional strain on the physiological mechanisms which control the functions of the body under the new and unusual conditions. Such additional strain will produce an unfavorable background for the tolerance of overloads, weightlessness and other flight factors.

In information published in "Pravda" on September 4, 1960, it was stated that these conditions were created by means of an air-conditioning system which was built into the capsule.

It is well known that animals consume oxygen and exhale carbon dioxide and water vapor in quantities proportional to the weight and surface of their bodies. In particular, the passengers of the second satellite, Strelka and Belka, inhaled 190-200 liters of oxygen per day, exhaling 150-170 liters carbon dioxide and 250-300 g water. To maintain the gas composition of the atmosphere in the sealed capsule constant, active

chemical substances were used which absorbed carbon dioxide and water vapor, and generated oxygen. These substances were placed in a special regenerative apparatus which controlled automatically the rate of absorption of carbon dioxide and water vapor and generated the required quantity of oxygen. By maintaining composition of the gas of the atmosphere constant the equipment simultaneously ensured constancy of pressure in the cabin.

In addition to carbon dioxide and water vapor, animals also exhale other substances which contaminate the atmosphere of the capsule and on accumulating in the air may prove harmful to the body. These substances include ammonia, hydrogen sulphide, volatile organic oxides, etc. Contamination of the air may also result from the operation of the apparatus and the equipment of the capsule. To purify the air from these harmful admixtures, special filters are provided in the regenerative system.

Data on the operation of the air-regeneration system and the state of the atmosphere in the cabin during flight are monitored by means of radiotelemetry which automatically transmits the necessary data to observation stations on the ground.

In addition to the above it is also necessary to maintain a given air temperature. Contrary to the views held by some people on the "absolute coldness" of interplanetary space the temperature in the capsule may vary from arctic cold to supertropical heat.

The temperature in spaceships which do not possess internal sources of heat depends on the properties of their surface, and on the capacity to absorb the energy of solar radiation and radiate solar heat into the surrounding space.

Depending on these properties, the surface of the spaceship can have a temperature as low as tens of centigrades below zero up to hundreds of centigrades above zero. Maintenance of the necessary temperature is complicated by the fact that the amount of heat from the Sun varies periodically when the satellite moves into the shadow of the Earth. Furthermore, it is necessary to take into consideration the periodical fluctuations in the quantity of heat released by the occupants of the satellite and the apparatus contained therein. These fluctuations are primarily associated with the daily periodicity of sleep and wakefulness of the occupants. Since the equipment for air-regeneration operates on "wastes" produced by the physiological functions of the animals, i. e., carbon dioxide and water vapor, the heat emitted by this equipment will also change in accordance with the level of the activity of the animals.

Heat from the cabin is removed by means of a liquid-air heat-exchanger (radiator) in which the excess heat is transmitted from the air to the circulating liquid which, in turn, gives up this heat in a special radiation heat-exchanger located outside the capsule.

Special equipment was used which enabled removal of the heat from the capsule at a rate which matched the rise in temperature during the various periods of flight so that the required temperature conditions were maintained in the capsule.

In the second Soviet satellite the animals (dogs) and the regenerative equipment were the basic source of internal heat supply.

Since their total heat release was equivalent to the heat released by a man, the creation of a system of temperature-regulation of the air in the capsule and analysis of its operation during flight are of great interest from the point of view of performing manned flight.

The processed data of the telemetric measurements carried out during the flight of the second Soviet satellite enabled an assessment to be made of the extent to which these problems had been solved during the actual flight. The percentage content of oxygen in the air of the sealed capsule was maintained continuously within the limits of 21-24 percent. The relative humidity during the first hours of flight increased gradually from 37-47 percent, probably due to the gradual introduction of the regenerative substance during operation. Later on, when the regeneration system was under steady-state conditions of operation, the air humidity was maintained at its initial level.

The air pressure of the cabin remained at the initial level without any appreciable fluctuations during the entire period of flight. The slight fluctuations in pressure at the beginning and end of flight could be explained by the changes in temperature and the percentage content of oxygen in the cabin air.

A discontinuity in the curves characterizing the pressure, oxygen content and the relative humidity between the 9th and 18th hour of the flight is due to the position of the orbit of the satellite; at that time the ground monitoring stations located on Soviet territory could not receive information from onboard the space ship.

During the first hours of flight a somewhat greater range of fluctuations and a higher temperature were observed which were due to the thermal conditions of the ship during the prelaunching period. (This satellite was launched on August 19, 1960). Later on, from the 8th to the 9th hour of orbital flight the temperature in the inhabited section of the satellite was stabilized and maintained within the limits of 16-19°C. Such narrow limits were not absolutely necessary during the flights of animals. However, in view of the proposed further development of space flights, it was considered advisable to aim immediately at creating conditions which were the most favorable for a human pilot.

The aim of the subsequent work was to improve further the systems for supporting life and testing their effectiveness from the point of view of its applicability to manned flight. In this respect, the flight of the third Soviet satellite was of great importance. Throughout the flight of this satellite necessary information on the operation of the entire equipment was obtained. These data are being processed and carefully studied.

## Feeding

In the life-support system of satellites the provision of food and water to the animals is, of course, of fundamental importance. However, it is difficult to take in food and water under conditions of weightlessness. On opening containers, water and food will move freely out of the containers and be inaccessible to the animals. To overcome these difficulties a method of supplying food and water in a combined form was adopted. According to this method a food mixture was prepared containing the necessary quantities of foodstuffs and water; by adding agar-agar this mixture was given the required viscous, gelatinous consistency. The mixture adhered to the walls of the container trough, even while it was turning over and under conditions of weightlessness. These containers were stored in automatic food-dispensers which, on receiving a signal from the experimenter, dispensed the combined food mixture.

The experimental animals were given a long, preflight training, during which they became accustomed to accepting this combined mixture from the automatic food-dispenser under conditions approaching flight conditions on the satellite.

During the flight of the second Soviet satellite, the dogs Belka and Strelka were fed morning and evening. On receiving a radio-command signal the container with the food mixture opened up to each of the dogs. The opening of the container and acceptance of the food by the animals were recorded telemetrically as well as visually on a television screen.

After the space capsule returned to Earth the operation of the automatic food-dispenser was checked and the contents of the containers analyzed. The automatic food-dispenser was in the normal operating condition. The first two containers contained 20 g (Belka) and 105 g (Strelka) of food, respectively; two more containers were empty, which obviously confirmed that the dogs had eaten the food.

Thus, Belka and Strelka were fed normally during their flight with the second satellite.

Physiological, Biochemical and Cytological Investigations

On November 3, 1957 for the first time in history a living being, the dog Layka, started on its long flight into space on board the second Soviet satellite. The flight lasted for several days and during it, extensive information was collected which confirmed the assumption of Soviet scientists that it was possible for the body to adapt to long-duration flights under conditions of weightlessness.

On August 19-20, 1960 a new biological experiment in space was made. The dogs Strelka and Belka flew for 24 hrs in the second Soviet satellite and then were returned to Earth together with the capsule.

In accordance with the program of the experiment, the following factors were investigated on the experimental animals during flight: pulse rate, electrical activity of the heart (electrocardiogram) in the pectoral region, the heart tone (phonocardiagram), arterial pressure, frequency and amplitude of the respiratory movements of the thorax, the temperature of the body at two points, the motor activity of the animals, etc.

The experimental data which was obtained enabled an assessment of the general state of the bodies of the animals under conditions of weightlessness over long periods to be made, as well as making a study of the compensatory reactions of the animals, etc. For two to three months before the flight the animals were under special observation. On the eve of the flight the animals were subjected to a careful veterinary and special examination and they were passed as being completely fit. At this period the pulse rate of Strelka was 63-69 per minute and of Belka 102-104. The electro- and phonocardiograms were typical for the particular breed of animal. The maximum arterial pressure of the dog Belka was 100 mm Hg, the minimum about 40 mm Hg.

The respiration rate of the animals varied between 15-70 per minute. The animals were quiet under the conditions of limited space in the sealed capsule of the artificial satellite. The body temperature of the dog Strelka was 38.0°C - that of Belka about 37°C, which was within the normal limits.

The results of telemetric measurements have shown that on the powered section of the flight considerable changes were observed in the functioning of the cardio-vascular and respiratory systems of the animals. Due to the effects of overload, noise and vibration the pulse rate of Strelka reached 160-180 per minute, respiration became superficial and frequent (150 breathing movements per minute). In Belka the changes in the physiological functions were similar but less pronounced. In particular, the pulse rate quickened to 100 to 140 per minute. The maximum arterial pressure increased to 190 mm Hg, whilst the minimum arterial pressure

remained almost unchanged. The respiratory rate of the animal was 110 per minute, reaching 240 for brief periods.

A few minutes after cessation of overload and vibration, the pulse and respiration rate dropped slightly for both dogs and about one and a half hours after weightlessness set in the pulse and breathing were comparable with those measured before the flight.

Under the subsequent influence of weightlessness, the basic physiological functions of the animals gradually returned almost to normal. During these conditions a slowing-down was observed of the pulse rate of the dogs to 74-112 per minute and the respiration rate to 24-26. Some weakening of the first and second tone of the heart was also observed. During weightlessness the characteristics of the arterial pressure of Belka returned almost to the initial level. The temperature of the body of both dogs at the end of the experiment was 37.3-37.5°C.

Of great interest are the results of investigation of the movements of the animal during flight. It was proposed to elucidate during the experiment the problem of the nature and state of the changes of the motor reactions of the animals under conditions of weightlessness. The broad formulation of the problem imposed specific requirements on techniques of the investigation. The dogs were able to move freely within certain limits. The dog was fastened in such a way that it could move consciously towards the food and fix itself in space only when its paws were unbent. In so doing, it pulled the ropes of the harness. On bending its paws, the dog lost contact with the floor and was suspended in air. Included in the harness ropes were contact potentiometric probes which recorded the force exerted by the paw. There were also probes which recorded the movement of the dogs towards the food. The movement of the dogs in the capsule was also monitored by means of two television cameras, one showing a side view and one showing the front view.

Analysis of the movements was made by comparing the television films with the information from the movement probes which was transmitted by the telemetry system and recorded in the form of curves. The latter made possible an objective evaluation of the movement seen on the films not only as regards amplitude but also as regards energy. As an example, a short description is given of the behavior of the animals on two characteristic sections of the flight of the second satellite. Thus, at the very beginning of the onset of weightlessness, the dog, suspended in air, tried to reach the floor with its paws and only when it felt support under its feet did it stretch its entire muscle system. After a short jerk the hind paws developed a constant static stress which fixed strongly the position of the dog. This proves that the dog preserved its rapid and adequate reactions to such an unusual stimulus as weightlessness.

Analysis of the television films and of the curves recorded from the telemetry information starting 12 hours from the beginning of the flight, under conditions of complete weightlessness, did not reveal any important disturbance in the coordination of movements of the dogs. The dog turned its head to its companion, sniffed at it, bared its teeth and barked. Due to absence of a support, its movements were not always rapid since the paws of the dog sometimes slid on the floor but the dog immediately corrected the error. Obviously, during such a short time it could not be expected that the various movements to be performed under a variety of situations would become quite automatic. However, a clearly pronounced tendency was observed to perform automatically certain simple and frequently repeated movements, for instance, the movements controlling the effort during fixing of the position of the body.

Immediately after landing, both dogs were subjected to thorough physiological examinations. The behavior of the animals did not differ from their usual behavior under laboratory conditions. They were active, they reacted adequately to the effects of external stimuli, they fawned, they reacted to their pet names, etc. The pulse rates of Strelka and Belka were 96-102 per minute, the respiration rates were 21-30 per minute and the arterial blood pressures corresponded to the initial values.

Immediately after the animals returned to the laboratory they were subjected to veterinary and special investigation (X-ray investigation of the thorax, biochemical examination of the blood and urine, etc.) These examinations did not reveal any appreciable changes.

Observation of the dogs over three months has shown that their flight in the satellite did not cause any unfavorable after-effects in the general state of the animals or their behavior. On November 30, the dog, Strelka, produced a litter of six puppies.

Observations have shown that the process of lactation in the dogs was quite normal. The appropriate unconditioned reflexes were fully conserved (licking of the puppies, specific reaction of the animal to the presence of strangers, unaccustomed separation from the puppies, etc.).

Data obtained during the second and third biological experiments in an artificial Earth satellite have confirmed convincingly the conclusion made after the flight of the dog, Layka, namely, that animals can adapt themselves to conditions of weightlessness over long periods. It is important that during conditions of weightlessness over long periods all the physiological functions (towards the end of the flight) should approach the initial level. This is of great scientific importance and permits sufficiently clear solutions of the problems connected with manned space flights to be derived. The results of the experiments prove that the equipment developed by Soviet scientists and engineers provides the necessary conditions for supporting life on long flights.

Undoubtedly, the results obtained from the biological experiments conducted in the Soviet satellite are unique. These experiments also show the reliability of the landing gear of the space ship. For the first time in the history of science living beings, orbited in a satellite, were brought back to Earth intact.

Another feature of these experiments was that an extensive program of investigations was carried out which included physiological-hygienic as well as biochemical, cytological, histological, microbiological and genetic investigations.

Study of the biological effects of factors associated with space flight and primarily of the biological effect of cosmic radiation is a complex and varied task, for the solution of which a variety of biological material and different methods of investigation had to be used on the second satellite.

The biological experiment in the satellite was preceded by extensive laboratory investigations on the preparation of the biological material, obtaining background information, study of the influence of individual factors which were reproduced in laboratory conditions (acceleration, vibration, etc.). Methods and techniques were evolved of ensuring the necessary conditions of living for biological material during a flight.

After returning to Earth the biological material was subjected to careful studies which are still continuing. The data obtained enable only preliminary generalizations to be made regarding the effect of various space-flight factors.

Investigation of the blood and urine of the experimental dogs, rats and mice has shown in the first instance that in the body of animals which are subjected to the effects of space flight conditions, shifts occur which are due to the so-called "stress-reactions". Several days after returning from a flight, the blood of the dogs Belka and Strelka showed a transient increase in the levels of $\alpha$-globulin, serum mucoid and total protein content, as well as a decrease in the cholinesterase activity. At the same time, no appreciable and constant changes could be detected in nucleic acid metabolism.

It is of interest that during laboratory investigations of the effects of acceleration and vibration certain biochemical changes were observed in these dogs which were comparable to the results obtained after the return to Earth of the animals from their flight. At present, further biochemical data are being collected which will enable the relative importance of the individual flight factors to be assessed.

Immunological investigations of the microflora and the bactericidal properties of the skin, and the phagocytic properties of the blood of the

dogs, Belka and Strelka, have shown that long-duration phasic changes occurred in the state of natural immunity, characterized by a lowering of the bactericidal properties of the skin and subsequent compensation and hydrocompensation for the disturbances in the reacting systems.

Analysis of the peripheral blood of the experimental dogs, and rats and mice has not shown any pathological changes in the white and red blood cells or in the hemoglobin content.

Cytological and histological investigations were also carried out on half-grown mice of both sexes (mongrel and pure mice of the line $C_{57}$Be). The haemopoietic organs, the central nervous system, the heart, lungs, etc. were studied. Compared with the controls, the experimental animals showed an increase in chromosomal aberrations of the bone-marrow cells. These changes were detected during the first days after the flight as well as during later periods. In view of this relatively specific nature of the damage caused to cells by ionizing radiations, it could be assumed that the above changes were due basically to the influence of cosmic radiation. However, the obtained results led to the more general assumption that the noted increase in the chromosomal aberrations of the bone marrow was caused by the overall effects of the various space-flight factors. Preliminary study of histological slices of various sections of the central nervous system, the spleen, the suprarenal gland, the liver and other organs did not indicate any pathological changes in the animals killed and examined during the first two weeks after return to Earth.

The investigations carried out so far do not reveal any appreciable genetic damage or physiological changes in the bacteria studied, alimentary tract bacillae of various strains, rod bacteria involved in butyric acid fermentation and staphylococci). Phages and cultures of cancerous Hela cells and skin flaps of man and rabbit which flew in space also differed little from their respective controls. Regrafting of the returned skin flaps was successfully carried out. Automatic bioelements (Translator's note: instruments which were developed specially for recording automatically the physiology and biochemistry of micro-organisms) with cultures of butyric acid fermentation bacteria functioned in space satisfactorily according to the planned program and the obtained data indicated that the bacteria were viable under conditions which were unusual to them.

It is well known that on the second Soviet satellite genetic investigations were carried out on a large volume of varied biological material.

The Soviet scientists R. L. Nadson and R. S. Filippov (1925) and the American scientist, Muller (1927) were the first to show that

ionizing radiations induce genetic changes in Drosophila flies and certain fungi. From that time onwards, numerous investigations made by Soviet and non-Soviet scientists have shown that various types of ionizing radiation - X-ray, $\gamma$-ray, neutron and others - are powerful means of bringing about genetic changes in all plants and animals, including Man. Hence, soon after the discovery of cosmic radiation, experiments were made designed to discover their genetic effects.

Soviet scientists were pioneers in these investigations. In 1936, test tubes containing Drosophila were placed in one of the stratostats launched in the Soviet Union. G. G. Frizen studied several generations of the progeny but did not detect hereditary changes. S. Pinkin and U. Sullivan of the USA, published in 1959 their results of experiments carried out with Drosophilae which flew for 30 hours in the gondola of an air ballon. Neither did they detect any hereditary changes in these insects.

However, these negative results cannot be considered final since in the above mentioned experiments the organisms were at an altitude not exceeding 23 km, where the pattern of the primary cosmic radiation differs considerably from that at higher altitudes. The genetic effect of cosmic radiation can be studied fully only under space-flight conditions. The beginnings of such investigations have been made by the experiments carried out on the second Soviet satellite.

It is well known that this satellite carried a number of species selected primarily for the purpose of genetic investigations: mice of two different strains; Drosophilae of two different strains, one of which had a very low, the other a very high, mutation rate (tendency to hereditary changes) under natural conditions; the spiderwort plant; varieties of pea seed; corn (maize), onion and nigella; actinomycete fungi - which have producers of antibiotic substances.

Immediately after landing cyto-genetic investigations were commenced with the purpose of detecting induced hereditary changes (mutations) in the above mentioned biological material. In addition, the influence of space-flight factors on the reproduction and development of these organisms was investigated. As only a short time has elapsed since landing of the second Soviet satellite these investigations are not yet complete. To carry out an adequate genetic analysis, at least two to three generations are required; for instance, for wheat, maize and peas, even if grown in hothouses, a considerable time is required. Only analyses of the genetic data from the rapidly reproducing species (Drosophilae and actinomycetes) are available; cytological analyses are not yet complete. From the other species (mice, plants and plant seeds) only data of the cytological investigations and observations on their reproductive rates and development are available.

What have these preliminary investigations shown?

Firstly, it can be generally stated that information of enormous importance has emerged. Space-flight conditions induce hereditary changes in various species and influence their development and reproduction.

The seeds of onions and nigella germinated much more quickly than the seeds of the controls. In the growing roots of seeds of peas, maize and wheat which had been in space, the mitotic rate was considerably increased. This increase in growth rate occurred particularly quickly in cultures of actinomycetes - which produce streptomycin. The growth of their colonies was accelerated sixfold.

As regards the genetic after-effects of space flights, the data can be summarized as follows.

For both Drosophila strains (those with low and those with high mutation rates under natural conditions) a slight but statistically reliable increase in the percentage of the dominant lethal mutations was observed (hereditary changes which appear in some of the first-generation progeny during the early stages of development); a considerably more pronounced effect was observed in the recessive sex-linked lethal mutations, which manifested themselves in a proportion of the progeny only from the second generation onwards. For both Drosophila strains, the frequency of appearance of these mutations was many times higher than in the controls. It is interesting that these phenomena were detected in the progeny which developed from embryos at various stages of development subjected to the effects of space flight. At present, cyto-genetic analyses are proceeding on the recessive lethal mutations in Drosophila which should reveal the nature of the chromosomal changes which have occurred.

No increase was observed in the percentage of chromosome recombinations of rootlets of onion and nigella seeds. In peas, maize and particularly wheat the cytological analysis of the cells of the growing points of the germinating roots revealed an appreciable increase in recombinations.

Thus, in various species stimulating as well as injurious effects of space flight were observed on the progeny and the processes of cell division in a generative and somatic tissue.

Primary cosmic radiation contains high-energy particles, the biological effect of which has not been clarified. It is known that protons have a much greater influence that X-rays or $\gamma$-rays on the genetic material. Therefore, in studying the effect of cosmic radiation on heredity, completely new and unexpected phenomenon should be anticipated.

In analyzing results it is necessary to bear in mind that in addition to cosmic radiation, other factors such as weightlessness, vibration, acceleration, etc. all exert their influence on the biological material. The effect of these factors on heredity has so far only been studied very little or perhaps not at all. It is known, for instance, that the combined effect of ionizing radiation and some other factors can change considerably the kind and the frequency of mutations. It cannot be ruled out that the detected genetic and cytological changes may be due to the combined effect of cosmic radiation and other factors that are specific to space flights.

The genetic effects discovered during the flight of the second Soviet satellite provided a basis for a completely new branch of science-space genetics. The obtained data indicate the potentialities of further genetic investigations associated with space flights. Determination of the genetic effects of space flights on some of the classical subjects of investigation (mice, Drosophila and certain plants) may prove very useful in assessing the biological dosimetry of the ionizing radiation which is encountered in space.

The revealed stimulating and mutagenic effect of space-flight conditions on various types of animals, plants and micro-organisms is of further importance since species of this type will accompany Man in his future space flights to ensure his oxygen and food requirement and perhaps solve the problem of the disposal of waste products of his metabolism.

Finally, investigations in this field will permit a better understanding of these space-flight effects and so enable the mechanism to be revealed by which disturbances in the structure of embryo cells cause hereditary changes of one type or another. If techniques can be worked out which will permit the differentiation between the influence of the individual factors (radiation, weightlessness, vibrations, acceleration, etc.) then the mechanisms causing genetic damage will be unravelled. This is one of the fundamental problems of modern biology.

Considering the entire material investigated so far, the conclusion can be made that no conclusive data have been obtained to prove that unfavorable biological effects are caused by radiation and other flight factors. However, it is necessary to bear in mind that these data are preliminary and relate only to the biological material, indices and methods of investigation applied in the experiments. A great deal of work has still to be done to complete the analysis of the available material. Furthermore, it is pointed out that the above conclusions apply only to specific flight conditions since the orbit of the second Soviet satellite was particularly favorable (it was located below the Van Allen radiation belt) and the duration of the flight was relatively short. It is also necessary to bear in mind that the intensity and composition of the

radiation in the radiation belts fluctuates continuously in time and space within extremely wide limits. Major work is necessary before final conclusions can be drawn on the safety of space flights from radiation effects.

The above mentioned physiological, biochemical, cytological, histological, microbiological and genetic investigations carried out during flight of the second Soviet satellite were continued in the experiments made on the third Soviet satellite.

## Investigations on the Third Soviet Satellite

In the same way as in previous experiments the basic medical and biological investigations carried out on the third Soviet satellite included:

1. The study of the biological effects of the main factors of space flight (overloading, long-duration effect of weightlessness, etc.) on living organisms;

2. The determination of the performance of life-support systems (regeneration, temperature-control, feeding, water-supply systems, etc.).

For solving medical and biological problems of space flights the applied methods and approach and the methods used are very important. In this respect, the experiments carried out in the third Soviet satellite are a new step in the investigation of cosmic space. In these experiments many new procedures, methods and biological materials were used appropriate to the biological material and the methods used. The radio-telemetry system provided very valuable information during a period of several days.

In the catapulted container, the sealed capsule of the satellite contained the two dogs, Pchelka and Mushka. This sealed capsule also contained five cages in which were two guinea pigs, two white laboratory rats, fourteen strain $C_{57}$ black mice, seven hybrid mice derived from SBA and $C_{57}$ and five mongrel mice. The capsule also contained six flasks with a high mutation strain of Drosophila, seven flasks with low mutation strains of Drosophila, six flasks with hybrid flies and two flasks with flies which were given with an additional protection of lead 5 g/cm$^2$ in thickness. The capsule also carried 5 g of pea seeds, 2 strains of wheat, 2 strains of maize, 4 strains of buckwheat and Windsor beans. A special container carried germinating onion seeds at various stages of development as well as moistened nigella seeds.

The satellite also carried several test tubes containing actinomy-eites-streptomycin producers; capsules with human tissue in culture both inside the thermostat and outside the thermostat; six test tubes containing chlorella in a liquid medium; further sealed ampules of a bacterial culture of the intestinal rod type K-12 and KK-12 (in oxygen and without oxygen) and two varieties of phages ($T_2$ and $T_4$) which were carried in ebonite cartridges. Also investigated were: a solution of deoxyribonucleic acid (DNA); a culture of Hela tumor cells, pulmonary and amniotic tissue of man, fibroblasts and bone marrow. In a special thermostatically controlled container and in an unheated container there were three automatic bioelements with cultures of the bacilli concerned with butyric acid fermentation and one "Bios" automatic container with the ova and sperms of a frog.

To study the biological effects of cosmic radiation the following selection of enzymes was carried on the satellite:

a) Pepsin, trypsin, alkaline phosphatase, catalase, peroxidase, ribonucease;

b) Homogenate of seed buds of wheat;

c) A preparation of cell nuclei (wheat);

d) A preparation of cytoplasm of cells (wheat);

e) Different strains (ordinary cyphomandra and plantain) of tobacco mosaic virus in aqueous solution and ammonium sulfate;

f) Influenza virus (cultured on different tissues).

In the process of preparing the experiment, methods of investigation were perfected as well as monitoring instrumentation. Also, an extensive series of laboratory experiments were carried out in which the influence of individual factors on the state of the living organisms was studied.

The dog, Pchelka - a two-and-a-half-year-old female - of light color with reddish spots, short-haired, weighing 5 kg 900 g, 33 cm high and 50 cm long.

The dog, Mushka - four-year-old, female, light color, fluffy, long-haired, weighing 5 kg 600 g, 25 cm high, 55 cm long.

Both dogs passed the preliminary examination, withstood the training and were subjected to pre-flight routine in good time.

For recording the functions of the animals during flight, a special set of medical instruments was developed.

During the flight, the following physiological indices were recorded by means of various sensors: electromyogram; electrocardiogram; heart tone; seismocardiogram; respiratory movements of the thorax; body temperature; motor activity of the animals.

The radiotelemetry system transmitted to the receiving stations on Earth the following data which is of considerable importance in assessing physiological changes: barometric pressure, temperature and humidity in the space cabin. Data on the functioning of the life-support system were similarly transmitted.

In this experiment, study of the movement of the animals which remained for a longer period under conditions of weightlessness was continued and extended. The investigations were carried out by means of television apparatus and specially developed equipment for detecting movement, electromyograms (electrical changes in the muscles). Simultaneous movements of the neck of the animal were also recorded. This comprehensive information enabled an assessment to be made of the nature, the degree of changes in the animals' movements and the time required for adaptation to occur to the conditions of weightlessness.

Much attention was paid to studying the state of the cardiovascular and respiratory activity of animals under conditions of prolonged weightlessness. It is known that following the increase in the general level of functional changes during the period of powered flight of the rockets and satellites, there will be a decrease in these changes when conditions of weightlessness set in. The problems relating to the times required for normalizing the cardiovascular and respiratory functions to return to normal after the effects of acceleration, and the specific nature of the influence of weightlessness, requires further detailed study. Due to the prospects of carrying out long-duration space flights, this problem is not only of theoretical but also of practical importance.

On the third Soviet satellite large quantities of biological materials were used, particularly microbiological and cellular. The selection of the specimens, the development and testing of methods for testing biological material to space-flight conditions are important links in medico-biological investigations of space flight. New techniques have been developed and biological specimens can be successfully used in subsequent experiments since they provide new and more extensive possibilities of investigation.

Thus, exposure of microbiological and cellular material to space conditions should permit studying the influence of space-flight factors to be studied not only on highly-organized complete organisms but at

the cellular and sub-cellular level. Application of micro-organisms and human cells in tissue cultures will probably enable investigating the influence of flight on elementary genetic processes to be studied.

A number of microbiological and cellular materials were flown into space repeatedly (intestinal rod KK-12 cultures, bacteriophage type $T_2$, Hela cancer cells, etc.) i. e., these cells had already been exposed to space conditions in the previous satellite and thus were exposed to space conditions twice. In some cases, the progeny of cultures which had been initially exposed to space-flight conditions were used. It is possible that such repeated exposure will enable physiological or genetic changes in cells to be detected which do not appear during a single exposure to space-flight conditions.

The enumerated number of microbes and cells used in the previous flight was substantially supplemented. In particular, besides the cancer cells in the tissue culture, normal cells of the human body were included; connective tissue fibroplasts; epithelial cells of respiratory tissue and human embryonic amniotic cells.

In spite of methodological difficulties, the use of tissue cultures is of great importance since it permits the influence of cosmic-space factors on cells to be studied which could not be determined in the actual body which possesses compensatory adaptations ensuring a rapid replacement of injured or dead cells by new ones. For this purpose a technique was developed which applies single-layer cultures on glass and permits the number of uninjured, injured and dying cells to be counted. The separation of the cells from the glass surface, changes in their staining characteristics and their ability to reproduce during subsequent cultivation were used in assessing cell injury and death. The technique of tissue culture also is valuable for studying genetic effects.

The third Soviet satellite also carried bone marrow of rabbits in a culture medium and two types of embryological specimens. It was intended to use these specimens for studying the initial stages of development of a fertilized egg, using, in particular, the ova and sperms of frogs. A special automatic device "Bios" (which has much in common with the bioelement AMN-1) was used to ensure mixing of the sperms and the ova (i. e., fertilization) at a predetermined time in accordance with the program of the experiment.

In addition to microbiological and cellular material, the third Soviet satellite carried deoxyribonucleic acid (DNA). This substance, which plays a major role in hereditary mechanisms, can undoubtedly be used to study the genetic conditions pertaining to space flights.

In the experiments with the third Soviet satellite lysogenic strains of intestinal rods (producing bacteriophages) were extensively carried out. Very small doses of ionizing radiation bring about a change in the genetic mechanism of the so-called lysogen bacteria, which manifests itself in alterations in the hereditary characteristics. In the experiments with lysogen bacteria, the ability of these bacteria to increase the production of bacteriophage in the culture medium was used as an elementary genetic test of the effect of ionizing radiation. Consequently, this is a particularly sensitive biological index for the given biological material. Furthermore, a part of the microbiological and cellular material was placed under an increased partial pressure of oxygen which is known to intensify their sensitivity to ionizing radiation.

Protecting some biological material (for instance, Drosophila flies) by means of an additional protective layer of lead will help in the investigation of those components of cosmic radiation which have the greatest biological (genetic) effect.

In the same way as in the earlier satellite, so-called AMN bioelements were carried, i. e., instruments which were developed specially for recording automatically the life function of micro-organisms. The AMN bioelements transmit to the ground information on the functions of microbes and so make possible the formulation of the necessary conditions for the existence of these living cells in regions of space where so far it has not been possible to introduce large animals which require considerable reserves of food and oxygen, depending on the flight duration.

In view of their very small dimensions and weight, these instruments can be fitted in large quantities on a single satellite and then switched-on, one after the other, so as to obtain the characteristics pertaining to various parts of the orbit. The information obtained by means of these bioelements carried on the third Soviet satellite (in a thermostat as well as in unheated containers) proved that they functioned satisfactorily in accordance with the experimental program. No deviations from the normal were observed in the function of these micro-organisms (bacteria-producing butyric acid fermentation).

Thus, the biological experiments carried out in the third Soviet satellite represent a new and important contribution to the development of space medicine and biology. As a result of these experiments, the following basic information was obtained:

1. The performance of a large number of systems designed to ensure the maintenance of life on spaceships was determined.

2. The influence of space-flight factors on a number of physiological indices was studied, many of these for the first time.

3. New methods were developed, tested and applied to a wide range of biological materials under space-flight conditions and much new information was obtained about the biological activity of some of this material.

The information accumulated so far indicates that the inclusion of biological specimens on satellites and spaceships can contribute to solving the problems which are posed as to the biological conditions that exist in various zones of cosmic space.

Various organisms can be used as test systems for the possible harmful effects in space. However, the appreciable differences in the sensitivity of these materials to that of the various organs and tissues of the human body must be borne in mind.

When interpreting the biological and genetic information so far obtained from the satellites, it is necessary to bear in mind that the organisms and external medium form an entity. Hence, it is important to aim not only at detecting the inherited changes that occur but also the purely adaptive phenomena which always accompany the process of evolution wherever it may occur.

Further biological investigations of cosmic space, with the resultant accumulation of new information and its correct theoretical analysis are important conditions for the successful solution of the extremely difficult and major problems facing space biology and medicine.

Summary

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The maintenance of life conditions is discussed with special reference to the second Soviet satellite. During the flight the proportion of oxygen in the air of the cabin could be maintained at 21 to 24 percent, whereas the relative humidity rose from 37 to 47 percent. The temperature ranged from 16 to 19$^{\circ}$ C. Water and food were provided together in a mixture solidified with agar in order to facilitate automatic dispensing in conditions of weightlessness. This was carried out twice daily by command signals from the Earth. Telemetric recording of the physiological parameters of the dogs, Belka and Strelka, during space flight showed the occurrence of tachycardia as a result of acceleration, noise and vibration, and there was also a rise in the respiration rate; a return to normal preflight values occurred during the condition of weightlessness. Movements of the animals were observed by television cameras and by strain gauge sensors mounted in the harness. No abnormalities were observed in the behavior of the animals after return to Earth or during the following 3 months. It was concluded from the experiments carried out in the second satellite that dogs could readily be accustomed

to space-flight conditions. Genetic changes were noted in the progeny of actinomycetes, plant seeds and fruit flies after return from space flight.

The third space satellite contained 2 dogs (Pchelka and Mushka), 2 guinea pigs, 2 rats, 26 mice, fruit flies, seeds and other biological materials which were included in order to study the effects of cosmic radiation. The results are not described.

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# PART III

## BIOLOGICAL EXPERIMENTS CARRIED OUT ON THE SECOND SOVIET SATELLITE

(Experiment with the Dog Layka)

## MEDICAL AND BIOLOGICAL INVESTIGATIONS CARRIED OUT ON SOVIET ARTIFICIAL SATELLITES

O. G. Gazenko, V. I. Yazdovskiy and V. N. Chernigovskiy

During the last decade Soviet and foreign scientists have carried out a number of experiments in satellites and rockets carrying animals into the upper layers of the atmosphere to altitudes of several hundred kilometers. The data obtained has enabled the nature of the biological phenomena which occur under conditions approaching those of space flight to be partially clarified. As a result of this it has become possible to study the influence on the body of factors which cannot be reproduced on the ground (weightlessness, types of solar radiation, cosmic radiation, etc).

The next step in this direction is to make use of artificial satellites. In contrast to high-altitude rockets which are used mainly for sounding the upper layers of the atmosphere, artificial satellites enable the behavior and the state of living organisms to be studied over long periods of orbital travel. Consequently, conditions can be created which, from the biological point of view, are near to, even if not exactly the same, as those obtaining in space flight. Carrying out experiments on satellites involve great technical difficulties. As in the case of high-altitude rockets, very rigid restrictions are imposed on the weight and the size of the equipment used and its consumption of electricity.

Furthermore, the recording and transmission of the readings of the instruments that monitor the behavior of the animal, and its hygienic and physiological parameters presented a very complicated problem until systems were developed for the safe landing and recovery of the animals and equipment carried in the satellite.

Biological investigations carried out in a satellite can be subdivided into two basic groups. Firstly, the study of the biological effects of space flight factors; and secondly, the development of equipment and control systems which would ensure satisfactory living conditions for the animal during all the stages of the flight of a satellite.

In contrast to biological investigations carried out on high-altitude rockets, artificial satellites permit a study to be made of long duration acceleration effects, noise and vibration effects during launching up to

the instant of guiding the satellite into its orbit, and the effects of a long period in the state of weightlessness which occurs during orbital flight. The influence of other factors on the animals, particularly of cosmic radiation, can only be studied on an adequate scale by means of satellites which can return their contained scientific equipment and animals to the earth.

It is understandable that carrying out long duration experiments will require equipment to be designed which is capable of automatically maintaining favorable conditions for life during the flight and also ensure the removal of waste products, etc.

The instrumentation should permit automatic and continuous recording of scientific data, and their transmission to ground-based receiving stations.

Finally, experiments of this type require special training of the experimental animals so that they become accustomed to various flight factors.

The efforts of the investigators were directed on the one hand to training the animals for the flight and assessing their vital activities, and, on the other hand, to cooperating with designers in developing the necessary life support system.

It was considered that for long duration experiments on the satellite, dogs would be just as suitable as they are for high-altitude rockets. However, in the case of rockets the experiments were of short duration, while in the case of satellites the animal would be required to remain for many days in a capsule of small size.

For the above reason much attention had to be paid to the preparation and training of the dogs so that they became accustomed to remaining in the confined space of the capsule over a long period. The training was gradual and based on the principle of applying successive steps. The animals were trained to wear special clothes, to defecate and urinate while wearing a sanitary device, and to remain in cages for increasingly longer periods, the dimensions of which were gradually reduced.

As a result of this training, the animals became accustomed to remaining quietly in mockups of the satellite capsule for the duration of the experiment, which lasted several days. Obviously, it was necessary to develop and repeatedly improve the harness and sanitary devices.

The requirements of strict economy in weight and size are reflected in the design and assembly of the pressurized capsule for the animal. The cylindrical metallic container proposed by the designers accommodated the animals and equipment in a compact manner.

Long and repeated testing of the design under laboratory and stand conditions enable this design to be used in the satellite. It is understandable that especially high requirements had to be met with regard to the hermetic sealing of the container that would protect the animal from the extreme rarefaction in the upper layers of the atmosphere.

The design and the scientific instrumentation of the capsule were closely related to the requirements of the physiological investigations. The experience gained with scientific apparatus on high-altitude rockets was extensively used. The apparatus was improved, and what is most important, was combined with a radiotelemetry system to ensure the transmission of the necessary parameters to ground stations. It was considered advisable to double wherever possible the sensors and the channels so as to increase the reliability of recording the physiological and hygienic indices.

Long duration experiments with animals have shown that the scientific instrumentation was adequately reliable and ensured the necessary accuracy of the recorded indices. The apparatus functioned automatically and was not controlled by the experimenter.

The feeding and watering of the dog presented complex problems. It was necessary to have accurate data on the composition and calorific value of the food ration required, the energy loss of the animal, and his water requirements. Several kinds of automatic food storage and dispensing equipment were developed and tested.

The preparatory work would have been incomplete without thorough study of the behavior and state of the animals during long laboratory experiments when the animals were placed into mockups of the pressurized capsule which were fitted with regeneration equipment, automatic food and water dispensers, waste-removal equipment and scientific apparatus. The results of these experiments proved satisfactory. The trained animals remained quiet for several days in the mockups of the capsule and no deviations from the normal could be detected in their basic physiology.

Furthermore, it was considered of great interest to determine the tolerance of these animals to some flight factors, particularly acceleration and vibration. It was assumed that an increase in tolerance would be achieved by placing the animal in the satellite in such a way that the longitudinal axis of the carrier rocket (direction of the acceleration) would be along the dorsoventral axis of the animal. It is well known that the greatest tolerance to the force of acceleration is achieved with (transverse) orientation. At the time no detailed data of the effect of transverse acceleration acting for several minutes was available in the literature. It was necessary, therefore, to carry out several series of preliminary experiments in which the most severe conditions were imposed, both as regards the magnitude of the acceleration and its duration.

Similar problems arose also during the study of the tolerance of the animals to vibration. In the first instance their tolerance to vibration was studied, as well as the reactions of the individual animals and any functional changes induced in them. The investigations carried out enabled information to be accumulated so that a comparison with the results obtained from the flight experiment could be made.

Summary

11688

A general discussion of the problems connected with the choice of the experimental animal and the logistics for the biological experiments in the satellite. The importance of careful design of the instrumentation and of the proper choice and thorough training of the experimental animals is emphasized.

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# PRESSURIZED CAPSULE FOR AN ANIMAL

A. M. Bakhramov and V. I. Yazdovskiy

As the altitude of ascent into the upper layers of the atmosphere increases, the conditions of the flight approach more and more those encountered in cosmic space. Even within the limits of the terrestrial atmosphere, extending, as is well known, up to the height of 1000-1200 km, the physiological conditions of flight are equivalent to those encountered in cosmic space (Strughold, 1954). This applies particularly to the barometric pressure and the oxygen content of the air. At a barometric pressure close to 47 mm Hg, warm blooded animals exhibit an altitude gaseous emphysema as a result of intensive vapor and gas formation in the liquid media of the body. Consequently, already at altitudes of about 19 km the total barometric pressure is insufficient to preserve the liquid media of the body in a normal state. For this reason conditions encountered at an altitude of 19 km with regard to barometric pressure are equivalent to those of cosmic space.

Although the percentage content of oxygen in the air is well maintained up to an altitude of 25 km and the gravitational stratification of the molecules starts only at higher altitudes, the partial pressure of oxygen falls progressively with decrease of the total barometric pressure. Theoretical and experimental data have shown that at a barometric pressure of approximately 87 mm Hg the respiratory function of an animal is interrupted, as the oxygen pressure in the alveoli of the lungs approaches zero. In this way, already at an altitude of approximately 15 km, the supply of oxygen to the body will be approaching conditions encountered in cosmic space.

The effect on the body of lowering the atmospheric pressure and oxygen insufficiency in the inspired air has been extensively studied; various means of protection have been suggested, in particular an oxygen breathing apparatus, scaphanders, pressurized capsules, etc. (Armstrong, 1954; Spasskiy, 1953; Yazdovskiy, 1953).

The concept of a hermetic capsule is not new; in one form or another it was suggested towards the end of the last century and at the beginning of the present by P. Ber, D. I. Mendeleyev and Kh. Shreter and others. However, only in 1931 did A. Piccard (1935) carry out his first flight into the stratosphere using a balloon with a pressurized capsule. Henceforth pressurized capsules were used by K. D. Godunov (in 1933), P. F. Fedoseyenko (in 1934), the brothers Piccard (in 1934), Steven and Anderson (in 1935) and others. Presently known pressurized cabins may be divided into three basic types: ventilational, regenerative and mixed (Rotatayev, Levashov, Yazdovskiy, 1957).

Ventilational pressurized capsules may be used during flights up to altitudes of approximately 25 - 30 km, when the compressor installations are still capable of creating the necessary pressure inside the capsule as a result of compressing the air of the external atmosphere (Fulton, 1948).

At greater heights the use of the external air becomes practically impossible in view of its great rarefaction. In addition, the compression of the air to the necessary ranges of pressure introduces the unwanted increase in the temperature of the air, which may contain injurious admixtures (ozone). In these circumstances it is more expedient to utilize for respiration a store of air carried aboard the flying ship. It is understood that the consumption of air, its escape from the capsule, etc., should be reduced to a minimum; this is achieved by the full pressurization of the capsule.

Due to the above mentioned causes, independent pressurized capsules with a closed regenerative air system should be used in cosmic flights (Simons, 1954).

The above considerations led to the choice of a pressurized capsule for animals in the second artificial earth satellite. In designing the capsule, the necessity for full and reliable pressurization was the most important factor. Equally rigorous requirements were presented by the weight and the overall dimensions of the capsule. With a minimum payload the capsule should be sufficiently strong to withstand the effects of vibration and overloads during the propulsive part of the motion of the satellite, and to withstand the increased internal pressure as well as the effects of meteoric material during the orbital flight.

The volume of the capsule was designed to take an animal, the instruments and apparatus, and also the corresponding installations for the introduction of electric cables and structural ducts for ventilation, air sampling, etc.

After designing various variants of the capsule, the one described here was selected.

## Brief Description of the Design of the Pressurized Capsule

The pressurized capsule for an animal (PCA) consisted of a cylindrical container of welded construction, 640 mm in diameter and 800 mm long, provided with a removable lid. The capsule was made of AMTsAM sheet aluminium alloy, 2 mm thick. In order to provide the necessary coefficients of attenuation and to give protection against solar radiation, the surface of the capsule was specially treated and polished.

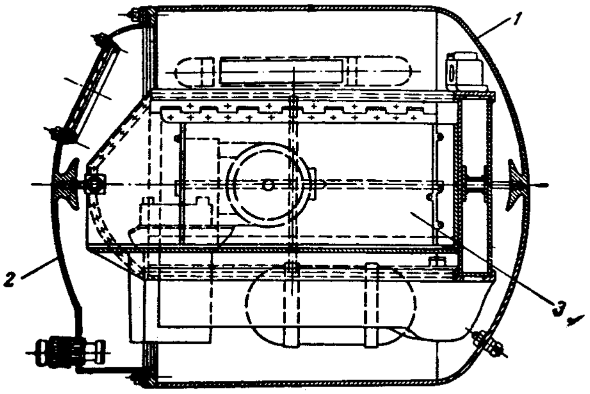


Figure 1. General Structural Diagram of the Pressurized Capsule for an Animal

1 - Shell of the capsule; 2 - Removable lid; 3 - Internal equipment

The general structural diagram of the pressurized capsule for an animal is shown in Figure 1, and certain structural details in Figure 2. As can be seen from Figure 2, the cylindrical shell 1 has protrusions 4, for fixing the capsule to a special frame by means of metal straps. The cylindrical shell has also in its front part a ring 8 with a slot, into which fits a rubber gasket 19, which secures airtight coupling of the main container with the lid of the capsule. For the attachment of the lid to the capsule, locking pins 12 were screwed into the ring of the cylindrical shell. The back end of the cylindrical shell had a base 2 welded to it, in the center of which was fixed a machined seat 17, with a fixed bush 18. The latter fixes the back part of the regenerative container. In the lower part of the base two hermetically mounted detachable connecting pipes 7 are fixed.

In order to observe the animal on the ground, the middle upper part of the detachable lid 3 carried a perspex porthole. The rubber washer 13 provides hermetic coupling of the porthole with the lid. Attachment to the cabin was carried out by means of locating pins. The porthole had a diameter of 160 mm and the thickness of the perspex was 5 mm. To the center of the lid a machined seat is welded which fixes the front part of the detachable equipment of the capsule by means of the fixed bushing 18. The lower part of the lid has a special pocket 5 for the three airtight connectors. To the front part of the lid is welded a ring 10 in which there are holes for the pins 12. The lid is tightened on the pins with nuts 11, which gives a complete pressurization of the capsule.

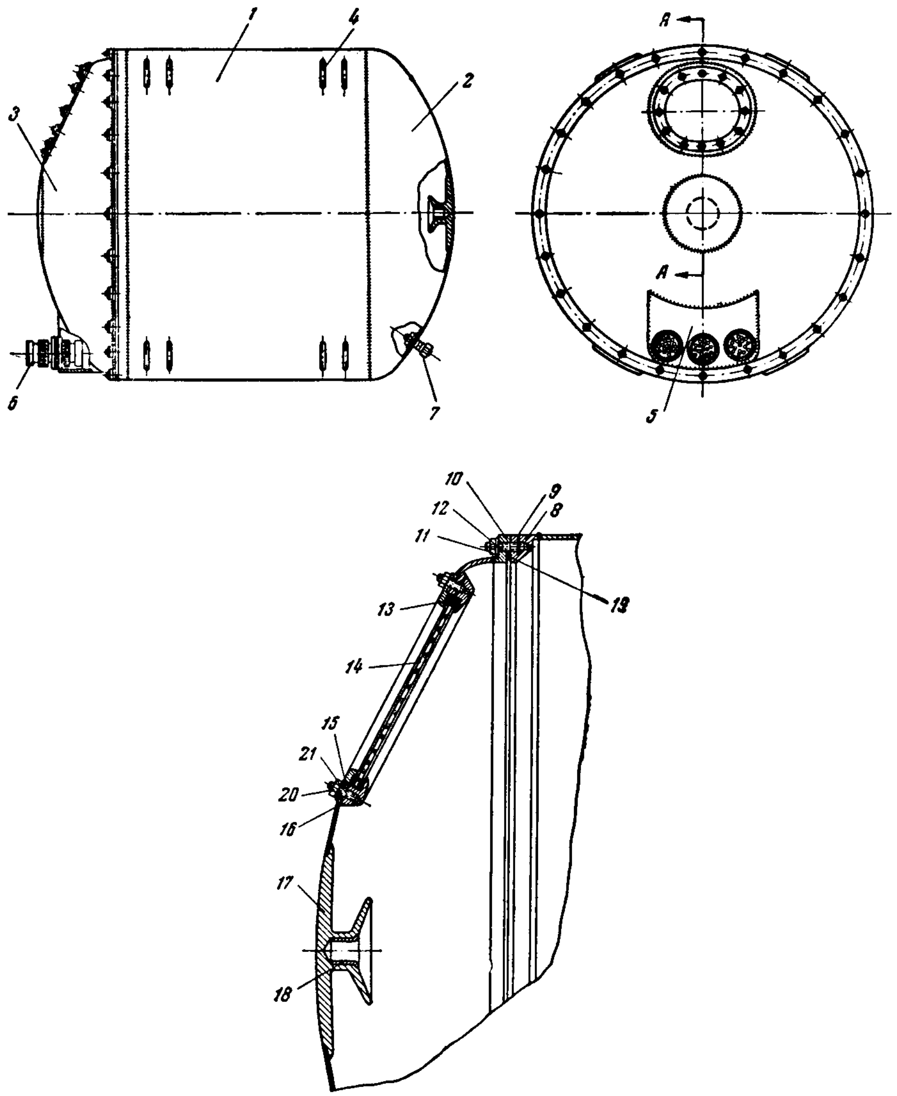


Figure 2. Diagram Showing the Construction of the Pressurized Capsule

1 - Cylindrical shell; 2 - Base; 3 - Lid; 4 - Ridges for mounting straps; 5 - Pocket for airtight connectors; 6 - Airtight connectors; 7 - Connecting pipe along the axis "A - A"; 8 - Rim of the shell; 9 - Locking pin; 10 - Lid flange; 11 - Nut; 12 - Pin; 13 - Rubber washer; 14 - Inspection window; 15 - Clamping ring; 16 - Porthole flange; 17 - Seats for fixing the regenerator box; 18 - Bush; 19 - Packing rubber ring; 20 - Nut; 21 - Pin

Tests of Pressurized Capsule for Animals

According to the described design, a number of capsules were made, each of which was subjected to static tests to withstand external and internal pressure and also for the effects of high and low temperatures.

Static Tests (Figure 3). With an internal excess hydrostatic pressure of 1 atm acting on the capsule for 1 minute, each capsule was subjected to a 100% operational load, the shell being carefully inspected at the end of the test. It was then exposed to an internal excess pressure of 1 atm with the operating load increased to 150%.

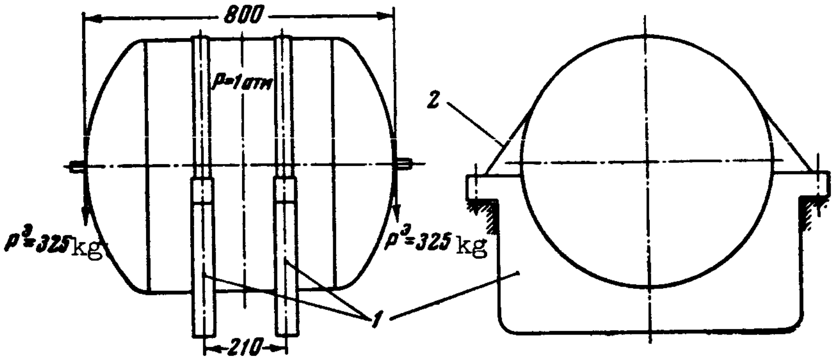


Figure 3. Diagram of Loading of Capsule During Static Strength Tests

1 - Holder; 2 - Strap

Tests for Resistance to External Pressure were carried out by lowering the pressure within the capsule. No capsule damage was observed as long as the rarefaction did not attain 0.1 atm. During tests for resistance to internal pressure over a period of 15 minutes the capsule was subjected to water pressure with the maximum pressure reaching 1.5 atm. Afterwards the capsule was inspected for airtightness in an alcohol bath, with an internal pressure of 1.3 atm. Similar tests were carried out when the capsule was filled with water under a pressure of 3 atm. No breakdown in the airtightness or any other damage to the shell was observed. According to the technical specifications the working pressure in the capsule should be 1.3 atm and the reserve strength factor should amount to 1.5.

The tests showed that the container of the capsule had sufficient strength and was sufficiently hermetic in the presence of external as well as internal excess pressures.

Testing of the capsule for airtightness and resistance to the effects of low and high temperatures was carried out with an excess pressure in the capsule of 1.2 atm. The capsule was in turn placed for a period of 12 hours in a chamber kept at a temperature of +30 and -20° C, where it was retained each time for a period of 20 minutes, after which it was checked for airtightness in an alcohol bath. Identical operations were carried out at temperatures of +40 and -30° C for a period of 15 hours, and at temperatures of +50 and -40° C for a period of 8 hours. The tests showed that temperatures in the range of +50 to -40° C have no effect on the strength or the airtightness of the capsule.

In this way the whole cycle of the tests to which the capsule was subjected showed that the selected design and technique of fabrication satisfies the main requirements of strength and airtightness of the pressurized capsule for animals.

## Internal Equipment of the Capsule

The internal equipment of the pressurized capsule for animals consists of installations for conditioning and regeneration of air, apparatus for the storage and supply of food to the animal, isolation of the intrinsic products of vital metabolism, installation for the placing of the animal itself, and other apparatus.

A metal frame was placed inside the capsule, to which were fixed a regenerator box, automatic machines, sanitary tank, instruments for studying the physiological functions, sensors and the experimental animals.

A riveted regenerator box of Π shaped form had a square cross-section (Figures 4, 5). The end of the frontal part of the box was covered by two cowls in the middle of which, mounted on special clamps, were fans with electric motors. From the point of view of its structure the box is divided into three channels; left, right and a transverse which joins the first two. The width of the left is 108 mm, and of the right, 118 mm. The regenerating substance is placed in the lateral channels. The channels are covered at the top by means of lids 2, which are detachable for convenience. A duralumin angle is riveted to the external walls, carrying self-locking nut 3 for bolting the lids.

The internal part of the Π shaped channel is braced with a sheet which connects the two channels and at the same times serves as the floor of the cabin. In the front part of the floor a square cut is made for

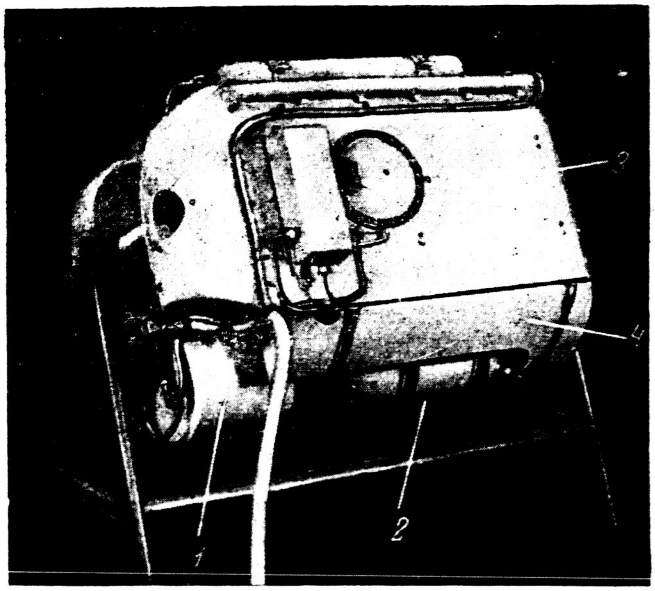


Figure 4. Internal Equipment of the Pressurized Capsule (view from the side)

1 - Automatic feeding machine; 2 - Water reservoir;
3 - Regenerator installation; 4 - Sanitary tank

the automatic feeding machine, and in the back part a circular cut for the pipe of the sanitary device 5.

The frontal part between the channels of the regenerator box carries a riveted transverse tube, to the center of which is attached a conical axle. The latter functions as a frontal point of suspension of the detachable equipment in the capsule. The rear point of suspension consists of an axle screwed into a thrust bushing fixed on the rear wall of the regenerator box. The floor is covered with a cork sheet 6, 10 mm thick, and part of the $\Pi$ shaped channel facing the animal is covered with 4 mm thick felt 7. Four special posts were attached to the lateral walls of the regenerator box; these posts were made of sheet steel (210 x 8 mm) and carried openings for fastening chains holding the animal in the capsule. All the joints in the regenerator box were hermetically sealed by means of sheet-rubber washers 1 mm thick.

A sanitary tank made of sheet aluminum alloy was attached to the lower part of the regenerator box by means of metal straps. In its rear part was drilled a hole to which was welded a pipe fastening the sanitary installation. The upper lid of the tank was fabricated to fit the lower part of the regenerator box.

One of the variants of the automatic food dispenser 8 was placed in an opening in the floor and together with it on its left side, facing the animal, was fixed a drinking bowl. The water was fed into the drinking bowl from the automatic water dispenser 10, placed on the outside wall of the regenerator box. A compressed air cylinder 11 feeding the water from the water reservoir into the automatic dosing installation was placed on the left lid. The water reservoir contained approximately 5 liters of water. The above lids were fastened: the automatic installation 12 for measuring the arterial pressure of the animal, temperature sensors 9 and cabin air pressure sensor 13, animal movement sensor 14, and also a block fixing the connections of the sensors (Figures 5, 6). The second variant in the assembly of the capsule equipment varied in that the feeding trough provided the animal with food and water together in the form of a jelly-like mixture.

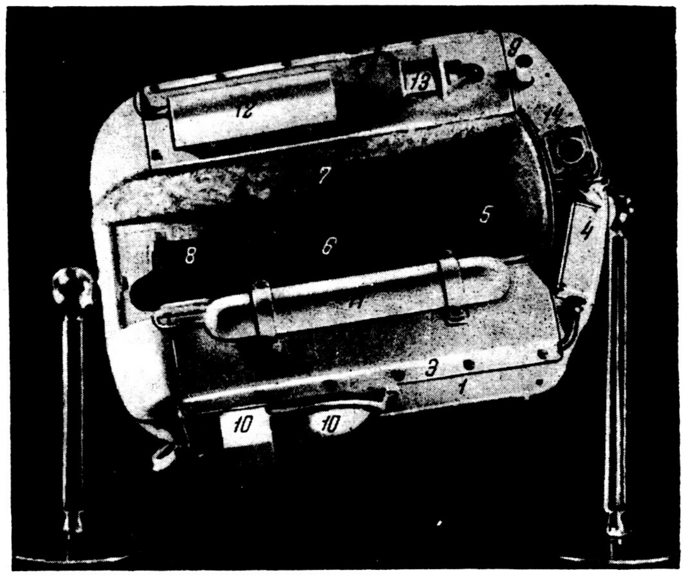


Figure 5. Internal Equipment of the Pressurized Capsule (top view)

1 - Regenerating plant; 2 - Regenerating box lid; 3 - Lid lock; 4 - Block for fastening sensor leads; 5 - Opening for pipe of sanitary device; 6 - Floor on which the animal was placed; 7 - Felt covering of the wall; 8 - Automatic food dispenser; 9 - Temperature sensor; 10 - Automatic water supply device; 11 - Air cylinder; 12 - Automatic device for measuring arterial pressure of the animal; 13 - Capsule air pressure sensor; 14 - Animal movement sensor

The fulfillment of the biotechnical requirements of the capsule, the performance of the equipment and that of the instruments, were all checked in extensive experiments in situ with animals. Certain modifications, additions and adjustments in the design of the capsule, in the layout of the instruments and equipment, were introduced in the course of the experiments.

These experiments have shown that the pressurized capsule fulfills all the fundamental requirements and secures conditions for normal living activities of an animal for a period of 20 days. There was every indication that the above described pressurized capsule for animals would prove useful for carrying out experiments on the second Soviet artificial satellite.

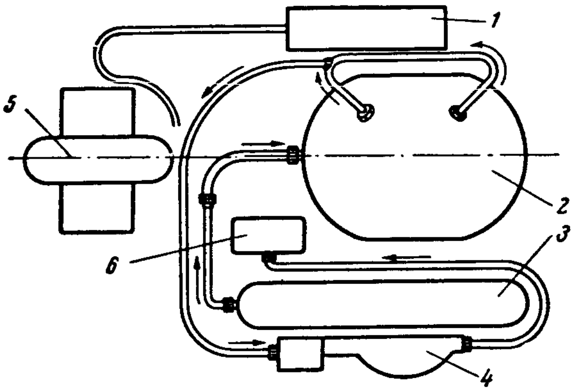


Figure 6. Principal Diagram of the Automatic Food Dispenser

1 - Automatic pressure installation; 2 - Small water reservoir; 3 - Air cylinder; 4 - Water dosing device; 5 - Automatic device with briquetted food; 6 - Drinking bowl

Immediately before the flight the animal was placed in the capsule and the sensors harnessed to it were connected to the amplifying equipment and the performance of all the equipment and registering instruments was checked; the lid of the capsule was closed and additional tests were carried out in a pressure chamber for airtightness. The capsule with the animal was then placed on the carrier-rocket of the artificial satellite. Subsequent observations on the behavior and general condition of the animal until the moment of launching were carried out through the inspection port of the capsule.

Checking of the capsule pressurization during flight was carried out by means of a potentiometric pressure sensor type MRD which was placed in the capsule.

During launching as well as during the orbital flight of the satellite, the strength and pressurization of the capsule were found to be sufficiently high.

Summary

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A sealed cabin for housing animals during space flights is illustrated and described. It consists of a cylindrical capsule, 64 cm in diameter and 80 cm in length, made of 2 mm aluminum sheet and provided with a detachable lid containing a plastic inspection port 16 cm in diameter. The capsule resisted excess internal and external pressures up to 1.5 atm without structural damage or leaking, and temperatures ranging from -40 to 50° C. The capsule is equipped internally with air-conditioners and regenerators, automatic feeding and other apparatus, supported upon a metal frame. It was possible to maintain animals, in a normal state of activity, in the capsule for 20 days. Author

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# RESEARCH APPARATUS

B. G. Buylov and R. G. Gryuntal'

Assessment of the condition of an animal in the pressurized capsule of an artificial earth satellite is based on the data which define the performance of the fundamental physiological functions. Thus, for example, the respiratory function is estimated by the frequency, amplitude and rhythm of the respiratory movements, from the quantity and composition of the inspired and expired air; the function of the cardiovascular system is assessed from the frequency and volume of the pulse, from the maximum and minimum arterial pressure, from the venous pressure, bioelectric activity of the cardiac muscle (ECG), etc. Of importance is the information regarding the state of the animal; whether it is at rest or in intensive movement with regard to the capsule, as well as data on the coordination of movements in the condition of weightlessness, on bioelectric potentials of the brain (EEG), and also information on the body and skin temperature of the animal, etc.

In order to assess correctly the data obtained regarding the physiological functions of the animal, it is necessary to consider the external medium, in particular the pressure, chemical composition and temperature of the ambient air, the capsule wall temperature; the magnitude of the gravitational forces increases by a number of times during the launching of the rocket, while they are practically absent during the inertial flight in orbit, and these must also be assessed. We must also assess the magnitude of the cosmic radiation, X-ray, ultraviolet solar radiation, etc.

In addition one also needs data on the performance of the automatic equipment which ensures the living conditions for the animal, issue of water and food at a given time and in the required quantity, the intake of water and food by the animal, the performance of fans in the capsule, and the voltage of the power supply sources used by the recording apparatus and the various automatic devices.

When an animal stays in the capsule of a satellite which is not equipped with means for return to Earth, information concerning the state of the animal and the environment is transmitted from the satellite by means of radiotelemetry and received and recorded in the ground stations. For this purpose shortwave and ultra-shortwave transmitters are used, since there are between the Earth and the satellite highly ionized layers of air which are impervious to long and medium radio waves. In view of the fact that short waves propagate in straight lines, radio communication of the satellite with Earth can be conducted only within the limits of direct visibility of the satellite. This time is limited to a few

minutes (Vakhnin, 1957). In order to increase the time of reception a number of telemetry stations, placed along the direction of the satellite orbit, are required.

In order to obtain more complete information, it is possible to employ on the satellite a memory system which will gradually store the information and transmit it at an increased speed during its flight over the receiving stations.

Transmission from the satellite of a great quantity of data is ensured by the use of a multichannel radio telemetering system. From the point of view of electrical energy economy, maximal noise immunity and the convenience of multichannel radio communication, telemetry systems with phase-pulse and code-pulse modulations are the most perfected (Yevdokimov, 1952). In the former the transmitted energy is radiated in the form of separate short pulses (of the order of a microsecond); the interval between the pulses usually exceeds by 100 to 1000 times the pulse duration. The transmitted signal, with this system of modulation, is characterized by the interval of time between the reference and the measuring pulses; the signals received by the telemetry station on the Earth are converted in such a way that the time interval is proportional to the voltage fed to the input of the registering oscillograph.

In the case of code-pulse modulation the energy is transmitted by means of equally short pulses, but the measured magnitude is characterized by the number and position of the pulses in every code group.

The recording of the physiological functions is in the form of a dotted curve, the distance between the dots being proportional to the frequency of interrogation of a given definite channel. The faster the rate of change of the curve, the greater should be the number of points per unit of time which depicts this curve. Conversion of the parameters of the physiological functions of animals and the environmental factors into electrical quantities is carried out by means of special sensors attached to the body of the animal or placed in the capsule. The sensing elements of the latter may be electric contacts, electrodes, variable resistors, photocells, photoresistors, piezo-electric crystals, etc. Feeding of the received electrical signals to the input of the radio telemetering system is carried out after conversion (amplification with respect to voltage and power) and switching. For this purpose it is necessary to use a special installation which works satisfactorily during automatic operation for a number of days. The main difficulty in achieving this requirement is the choice of the method of registration and the positioning of the sensors on the body of the animal who is in the satellite in a relatively unconstrained state. The apparatus should work reliably in conditions of gravitational overload and the considerable vibration occurring during the launching of the satellite, and also in conditions of weightlessness during orbital flight. In addition, the apparatus mounted in the

satellite should have minimum dimensions and weight, be economical with respect to electrical energy consumption, and have a high stability against mechanical and electrical disturbances. As a source of electrical energy, chemical or solar batteries, or a combination of both, are used. From chemical sources the greatest specific energy content (amount of stored electrical energy per kg of weight) is shown by silver-zinc battery for purposes requiring a high current and low voltage, and by oxygen-mercury batteries for high voltage and low current consumption. Semiconducting converters of voltage may also find wide application, since they will permit considerable reduction in the weight of the electrical supply sources. Solar batteries are also very promising; a silicon battery may yield from 1 square meter of surface up to 150 watts (Chechik, 1956).

Conversion of the animal physiological function parameters into electrical quantities and their subsequent transmission to Earth may be carried out in various ways. For instance, the frequency, rhythm and relative change in the depth of respiration may be registered by means of a thermistor placed in the stream of inspired and expired air (Henry et al., 1952). The resistance to the passing current will change in the sensor according to its temperature, which in its turn will change according to the rhythm of respiration. The disadvantage of this method lies in the difficulty of mounting the sensor in the stream of expired air when the animal is without a face mask and in a relatively unrestrained state which permits movement of the head. The sensitivity of the sensor falls when the temperature of the medium approaches the temperature of the expired air. The latter disadvantage is eliminated by using thermistors heated, by passing current, to a temperature above that of the expired air. However, in this case the registration of the function is distorted, since the sensor will be cooled by inspired as well as by expired air. Using surgical means, it is possible to place a small semiconducting thermistor into the frontal sinus connected with the respiratory passages, but experiments have shown that sensors so placed were rapidly covered with organic matter and then failed to work.

The simplest and most reliable means of converting the frequency and rhythm of respiration into electrical quantities is by means of potentiometric sensors registering changes in the circumference of the chest during respiratory movements. From such a sensor may be obtained a sufficiently strong signal which does not require further amplification and conversion, and is suitable for transmission to the input of a radio telemetering system. The disadvantage of this method is due to the disturbances taking place when the animal moves.

Blood pressure in an anaesthetized animal is registered by means of a cannula and a Statham pressure converter (Henry, 1952). The blood pressure of an anaesthetized animal which is left in a relatively unrestrained state can be determined by the oscillation method (Savitskiy, 1956). The blood vessel in which the pressure is measured should be situated

sufficiently near the surface of the skin so that it can be constricted by the cuff. The air is fed into the cuff of the sphygmomanometer from a special cylinder through a reducer and electromagnetic valve, regulated automatically by a relay system from a piston compressor with electric actuation, changing the pressure in the cuff according to a given program. The pressure in the cuff is registered by means of a manometer with a potentiometric sensor, and is conveyed along a separate channel to the radio telemetering system.

The sensing element of the oscillation sensor receiving the oscillations of the walls of the vessel and converting them into electrical voltage is usually a piezoelectric crystal or a thermistor, which is heated

by the current passing through it to the temperature of 100-200$^{\circ}$ C. In the latter case the pressure oscillations in the sphygmomanometer cuff caused by the oscillations of the walls of the vessel are utilized; the changes in pressure cause changes in the density of the air stream which flows past the heated thermistor. In this way, a periodic change of voltage is produced in the thermistor, which follows rhythmically with the pulse oscillations of the walls of the vessel. The voltages obtained from the oscillating piezoelectric and photoelectric sensors or thermistors are very low (of the order of 1 millivolt), and in order to feed them to the input of the telemetering system they must be amplified. Amplification is necessary also for the electrical voltages fed from the electrodes for the registration of the electrocardiogram, electroencephalogram and the electromyogram. Animal body temperature sensors or sensors of the ambient air are usually in the form of a wire or semiconducting thermistor, also frequently requiring amplification of their signal.

The most complete picture of the behavior and state of an animal in the capsule may be obtained by means of periodic filming, with subsequent return of the film cassettes to Earth, transmission of the picture from within the capsule by a method similar to the transmission of pictures by facsimile telegraphy, or by means of television. However, application of the above means requires sufficiently powerful sources of electrical energy. Incomparably more economical and not less suitable is the utilization of movement sensors registering over a period changes in the coordinates of one of the points on the body of an animal with respect to the three coordinate axes, which gives a representation of the movement of only one particular part of the body of the animal. If as the point of registration is chosen, for instance a part of the head, and sufficient material is collected about its movements during the feeding of the animal under laboratory conditions, then by means of comparison it is possible to estimate to some extent the disturbances of coordination of such movements under the action of weightlessness. The sensor may be for example in the form of a miniaturized electric bulb fixed on the head of the animal and three photocells or photoresistors placed on the walls of the capsule at a certain distance from each other. The current passing

through the photocell or the photoresistors will be a function of their illumination, and the illumination of each photocell will depend on its distance from the bulb, that is on the coordinates of the bulb.

The practical realization of the above method is associated with considerable difficulties, since it is necessary that:

the luminous flux of the bulb should be uniform in all directions;
that the walls of the capsule do not reflect any light;
that there should be no other sources of light in the capsule during registration;
that there are no obstacles in the path of the luminous flux.

It is very difficult to secure the bulb reliably on the head of an animal without causing it certain painful sensations. Similar sensors but working on a mechanical variant and registering coordinates of one of the points on the body of an animal by means of three potentiometric sensors are also inconvenient, inasmuch as they require attachment to the animal of threads which may be damaged by the animal. The simplest and most reliable method of registering the movement of an animal is to use one potentiometric sensor; however, this method does not allow assessment of disturbances in coordination.

For registration of physiological functions and movements of the dog Layka on the second Soviet artificial satellite, a special series of medical instruments were constructed which included an amplification and switching unit with two amplifiers, a set of sensors, current supply sources and various mechanical attachments, the latter being used only under laboratory conditions (Figures 1, 2).

According to the program the recording of a number of physiological functions was envisaged:

frequency of cardiac contractions (electrocardiogram);
frequency of respiration by measuring the perimeter of the chest;
magnitudes of the maximal arterial blood pressure by means of the oscillation method.

The motor activity of the animal was recorded by means of actography with the help of a movement sensor. For the registration of the heart potentials, silver electrodes were used in the form of small rings 5mm in diameter, implanted into the soft tissue of the dog's thorax. The voltage from the electrodes was amplified by a three-stage electronic amplifier of type UES-02F and was fed to the input of a radio telemetering system. The coefficient of amplification was of the order of 3000. The frequency and rhythm of respiration (Figure 3) were registered by means of "tensolite" rheostatic sensors in the form of small strips carried on the thorax of the animal. The sensors, which had a resistance from 300 ohm

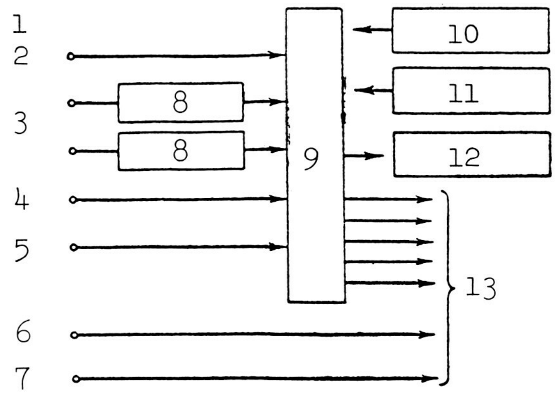


Figure 1. Block Diagram of the Recording Apparatus

1 - Sensors; 2 - Respiration; 3 - Electrocardiogram; 4 - Pressure in the sphygmomanometer cuff; 5 - Movement; 6 - Temperature in the capsule; 7 - Pressure in the sphygmomanometer cuff; 8 - Amplifier; 9 - Switching unit; 10 - Programming device; 11 - Supply sources; 12 - Automatic device for pressure in the sphygmomanometer cuff; 13 - To the radiotelemetry system

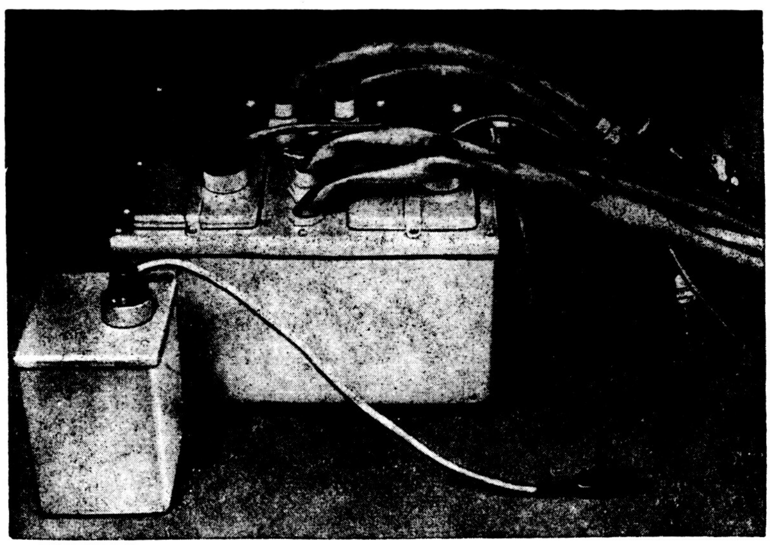

Figure 2. Amplifier-Switching Unit, with Two Amplifiers, Showing in Front a Separate Amplifier

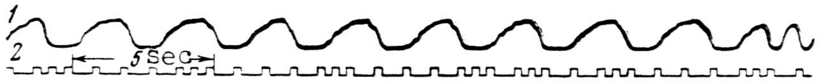


Figure 3. Record of Respiration

1 - Respiration; 2 - Time marker

to 20-25 k ohm, were connected into one of the arms of a potentiometric circuit. To improve the utilization of the system, two sensors were connected in parallel to the measuring circuit (Figure 4).

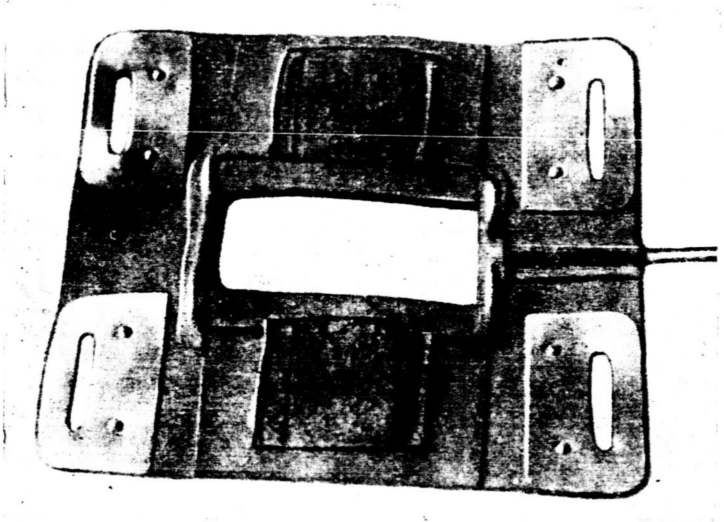

Figure 4. "Tensolite" Respiration Sensor

The maximal arterial pressure was determined by means of an oscillation sensor (Figure 5). A PEK-55 piezoelectric crystal converted the oscillations of the carotid artery wall into voltage oscillations. The oscillations channel amplifier type UES-02F was similar to the amplifier of the electrocardiogram channel and was installed in the same amplifier-switching unit. The automatic device creating the pressure in the sphygmomanometer cuff consisted of a cylinder in which the volume of air (and consequently the pressure of the air) was varied by means of a piston actuated by a small electric motor according to a given program. The pressure in the cuff, converted by means of a potentiometric sensor into electric current, was transmitted through the (an)other channel (Figure 6).

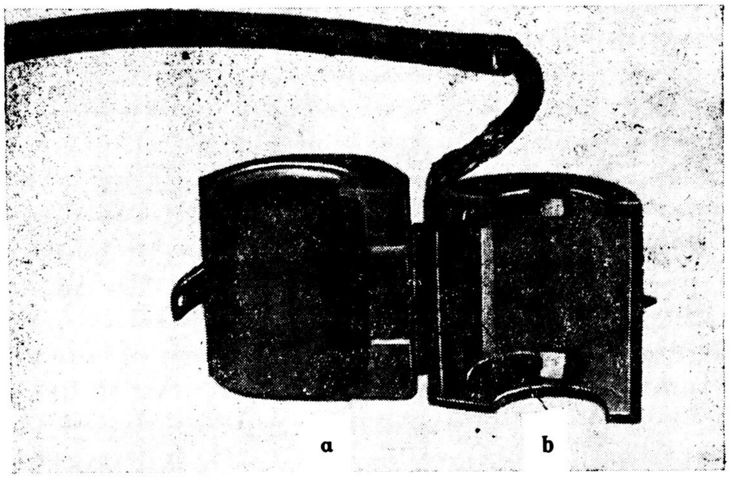


Figure 5. Open View of the Oscillation Sensor

a - Rubber cuff of the sphygmomanometer;
b - Piezoelectric crystal

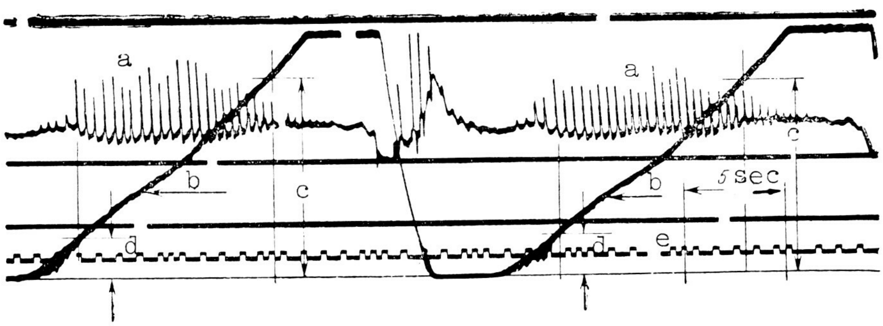


Figure 6. Recording of the Arterial Pressure

a - Oscillations; b - Pressure of air in the cuff; c - Maximal arterial pressure; d - Minimal arterial pressure; e - Time marker

The duration and amount of the animal's movements were registered by a potentiometric sensor, using a wire wound variable resistor connected with a polycaprolactam thread reeled on a drum with a spring; the sensor was located on the rear wall of the capsule (Figure 7). The other end of the thread was fixed to the harness of the animal; during movement of the

latter, due to the changes in length of the thread, the variable resistance of the potentiometric sensor was changed.

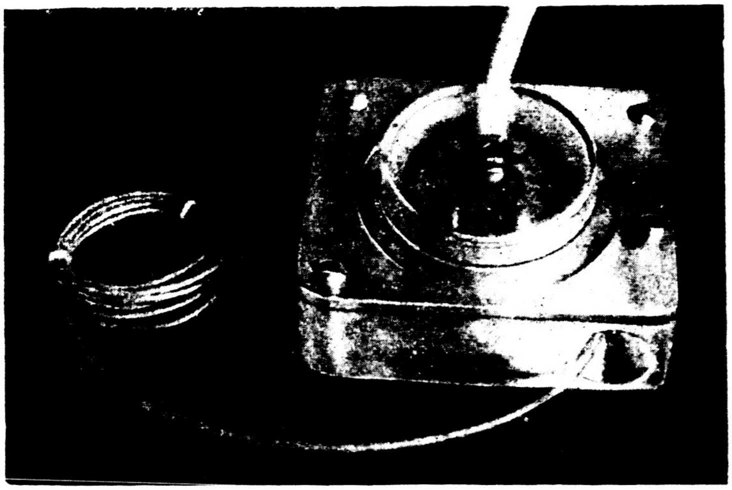

Figure 7. Potentiometric Pressure Sensor

The air temperature in the capsule was recorded by a wire-wound thermistor and the air pressure by a potentiometric pressure sensor. Command pulses of control and power supply voltages from special batteries were sent to the amplifier switching block, from which were also taken output parameters for transmission to the radiotelemetry system.

In order to utilize the power supply economically, the recording apparatus in the satellite worked only periodically, due to a special programming apparatus.

The above described apparatus, as is well known, ensured registration of the necessary physiological and hygienic parameters during the flight of the satellite. The "electrograms" received on ground-based telemetry stations proved satisfactory and allowed estimation of the state of physiological functions of the animal and hygienic conditions of the environment during various phases of the flight. It can be concluded that the set of medical instrumentation worked satisfactorily during the flight.

Conclusions

1. Analysis of the electrograms received from the satellite (physiological and hygienic parameters) shows that the apparatus worked satisfactorily, and the chosen methods of study responded to the requirements of the experiment.

2. The necessity for further development and broadening of methods of research was revealed. Particularly promising are the use of apparatus for the transmission of pictures of the animal and also bioelectric methods of investigation.

Summary

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The methods used for recording physiological data in the dog, Layka, during the second space flight are described. The electrocardiogram was picked up by silver electrodes implanted in the chest wall and amplified 3000-fold before transmission. Respiratory movements were picked up by two strain gauges surrounding the chest and connected in parallel. Arterial blood pressure was recorded by a piezoelectric element applied to the carotid artery; an occluding cuff was periodically inflated and deflated by a piston, the internal pressure being recorded potentiometrically through a second channel in order to determine the cutoff pressure by the usual sphygmomanometric procedure. Movements were recorded by means of a spring-loaded thread attached to the animal and running to a variable resistance. The temperature and pressure of the air in the cabin were also recorded. An amplifier pack was provided to handle the telemetered information and incoming command signals. The system gave satisfactory service during the flight of the dog, Layka, in the second Earth satellite.

Author

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# AIR REGENERATING SYSTEM IN A PRESSURIZED CAPSULE

A. D. Seryapin

Important note: Although the author describes in detail the reasons why a chemical method of regeneration was used in the satellite and gives information on the performance, the actual compounds used are not specified.

In order to insure for an animal, satisfactory conditions during flight in an artificial Earth satellite lasting for a number of days, it was necessary to provide an atmosphere of a definite composition and pressure. In the artificial Earth satellite this problem was solved only by the use of pressurized capsules of the regenerative type. The air regeneration system must insure the absorption of carbon dioxide and moisture expired by the animals and generation of the necessary quantity of oxygen.

At the present time a number of methods of air regeneration have been submitted; these may be reduced to three fundamental types, based on:

a) utilization of chemical substances to absorb carbon dioxide gas and water vapor with an additional system of oxygen enrichment of the air;
b) use of chemical reagents which, in addition to absorbing carbon dioxide and moisture, generate the necessary quantity of oxygen;
c) biological regeneration of the air, utilizing various forms of aquatic plants, algae and microorganisms.

In the regenerative cabins of experimental aeroplanes and stratospheric balloons (Spassiky, 1938, 1939, 1940; Apollonov, Mirolyubov, 1937, 1938; Mirolyubov, Apollonov, 1938; Brestkin et al., 1934) soda lime in the form of large granules (ChAL - chemical lime absorber) was used for the absorption of carbon dioxide. One kilogram of such absorber absorbs 150 grams of carbon dioxide.

Silica gel with an admixture of calcium chloride was used to absorb water vapor from the air. One kilogram of this agent absorbs 350-400 grams of moisture.

In certain cases motors with fans, and in others injectors, were used to impel an air current through cartridges containing the absorber. To replace the oxygen consumed, liquid oxygen was also used; one liter of the latter yields, on evaporation, approximately 800 liters of gaseous

oxygen. Alternatively, gaseous oxygen compressed in steel cylinders to a pressure of 150 atm may be used, in which case it is utilized simultaneously for injection.

Calculations showed that the above variants of regenerative plants are unsuitable for use in an artificial satellite capsule due to their weight and size. A dog, weighing 5-6 kg, requires for one hour an average of 4-6 liters of oxygen, and expires approximately the same quantity of carbon dioxide and 6-8 grams of water vapor. It is easy to calculate that in order to absorb the carbon dioxide and water vapor expired by the animal during a ten-day journey in the pressurized capsule, 5-6 kg of silica gel and 9 kg of the above carbon dioxide absorbent would be required. It is necessary to allow also for the expenditure of gas (oxygen, air, etc.) for the work of the injector. If we assume this expenditure equal to 1.25 liters per minute, then the weight of the equipment for the regeneration of air during a ten-day experiment will amount to 160 kg.

Instead of using an injector, a fan with a motor may be used as an impeller, to circulate the air through the regeneration plant, and liquid oxygen may be used for replacing the oxygen consumed by the animal. However, the fan consumes electrical energy, and storage of liquid oxygen requires the use of special liquid oxygen apparatus of the type KPZh-30.

The use of liquid oxygen introduces additional difficulties. During its storage in apparatus, even in the idle state, there is an intrinsic evaporation of oxygen (70-90 g/hour) which causes an unproductive expenditure and a dangerous increase in the concentration of oxygen in the air of the capsule. It is clear that the above system will also have considerable weight and dimensions.

The biological method of air regeneration is still being developed. Practical utilization of this method requires a large capsule and sufficiently suitable conditions of illumination and temperature. As calculations have shown (Bowman, 1953, 1954), utilization of this method will prove expedient only during flights of very long duration.

Thus, the above described methods of insuring normal conditions of life for the animal in a pressurized capsule of an artificial Earth satellite cannot be considered fully satisfactory.

A problem of developing an air regeneration system has arisen, which would absorb per unit of weight and volume sufficient quantity of carbon dioxide and water vapor and generate simultaneously a sufficient quantity of oxygen. In solving this problem it is necessary above all to determine the permissible concentration of carbon dioxide and oxygen in the inhaled air and the optimum conditions of barometric air pressure in the capsule. Since the capsule had a considerable reserve of strength it

was possible to create within it barometric pressure equal to the atmospheric pressure.

Numerous Soviet and foreign authors (Apollonov, Mirolyubov, 1937, 1938; Mirolyubov, Apollonov, 1938; Spasskiy, 1938, 1939, 1940; Annin, Zburzhinskiy, 1938; Brestkin et al., 1934; Sirotin, 1952; Prayor, 1930; Strughold, 1954, and others) established that in a small pressurized capsule the concentration of carbon dioxide should not exceed 1-2 percent, and the oxygen content at the standard barometric pressure should be 20-40 percent. These data served as the basis in the formulation of the medical requirements which would provide satisfactory living conditions for animals during a prolonged stay in a pressurized cabin.

As regenerative substances special highly active chemical compounds were used which, when reacting with water vapor and carbon dioxide, evolved sufficient quantities of oxygen for the breathing of the animal. These substances had additional properties, namely they were also absorbing certain noxious gases forming during the metabolic processes of the animal. Artificial ventilation derived from two small electric motors with fans was provided to secure good contact between the air and the regenerative substance.

For calculating the necessary reserve of the regenerative substance, results of the preliminary tests were used. These showed that animals weighing 5-7 kg, when at rest and in optimal temperature conditions, evolve 3 to 3.5 liters of carbon dioxide and require 4 liters of oxygen per hour. When due to temperature rises in the capsule or due to other factors the animal becomes restless, the oxygen requirement and the subsequent evolution of carbon dioxide and moisture will increase. For that reason the reserve of the regenerative substance was taken on the basis of an average oxygen requirement of 5 liters per hour.

The experimental data were also used in calculating the rate of ventilation with respect to carbon dioxide. If we accept that the maximum permissible concentration of carbon dioxide in the cabin should not exceed 2 percent, and its content at the output end of the absorbing installation should be substantially zero, then the rate of ventilation should be not less than 350 liters/hour, i.e. 5.8 liters/min. The required volume of ventilated air was found in almost all experiments to be slightly greater than the calculated value, which necessitated maintaining a definite quantity of moisture in the air of the capsule. As a result, the percentage content of carbon dioxide varied within 0.1-0.5 percent.

The use of chemical substances which absorb moisture and carbon dioxide with a simultaneous evolution of oxygen is very economical when compared with other systems. For comparison, the weight data of the three types of installations used in 10 days' stay of an animal in a capsule are given.

If the weight of the installation using the highly active chemicals be taken as a unit, then:

1) installation employing chemical lime absorbers, silicagel and gaseous oxygen will weigh 8;
2) installation using chemical lime absorbers, silicagel and liquid oxygen will weigh 3.5.

The above comparison once again confirms the advantages of using the regenerating system absorbing carbon dioxide and moisture with simultaneous evolution of oxygen.

The supplies of the regenerative substance were placed in a special II-shaped aluminium container of 1.5 mm wall thickness. The installation consisted of two units (left and right) which were connected at the rear by a tube. In the front parts the units carried diffusers in the middle of which openings were made to accommodate the motors with fans. At the top the units were covered with hermetically sealed lids.

One to two hours before the experiment commenced the regenerating substance was weighed and a sample taken for oxygen and carbon dioxide analysis. Then the substance was placed in the installation and the volume of the processed air determined by taking readings at the inlet and outlet using a Buylov electroanemometer. The latter instrument permits measuring the volume of air and its rate of flow up to 0.5 m/sec, in a duct from 1 $cm^2$ cross-section upwards. The temperature and relative humidity of the air in the capsule were determined during the experiments by means of a barothermopsychrometer. In addition, temperatures at various points in the capsule including the inlet and outlet of the ventilating installation were measured by means of temperature sensors to an accuracy of $\pm$ 0.5° C. The whole apparatus was calibrated before the experiment, using standard instruments. At six-hourly intervals throughout the whole experiment hygienic indicators of the medium were registered and air samples taken for oxygen and carbon dioxide content analysis. A Holden air analyzer was used for this purpose. On completion of the experiment the regenerating substance was reweighed. The analysis of the substance for oxygen before and after the experiment was by means of the volumetric method, while that for carbon dioxide was gravimetric.

Experiments testing the regenerating system were carried out on dogs weighing 5-7 kg. According to the direction of air flow in the regenerating installation the experiments were divided into three main series.

The disposition of the substance and the flow of air in the experiments of the first series are shown in Figure 1a.

As may be seen from the diagram the ventilation was carried out simultaneously by both motors; one of them impelling and the other expelling.

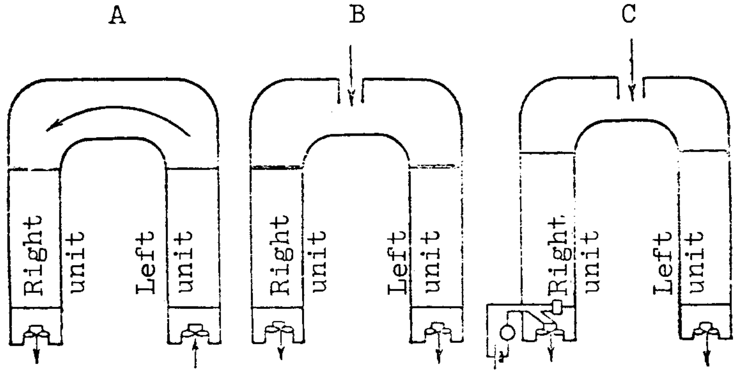


Figure 1. Diagram Showing the Distribution of the Regenerating Substance and the Airstreams in the Regenerating Installation

A - 1st Series of Experiments; B - 2nd Series; C - 3rd Series

The hygienic characteristics of the microclimate in the capsule during one of the experiments is shown in Figure 2. The supply of the regenerating substance with respect to oxygen was in this case taken to allow for a 20 days' stay of the animal.

From the data in the graph it follows that the oxygen content of the capsule was 19.5 percent at the beginning of the experiment and increased within a few days to 48 percent. During that time, for certain technical reasons a porthole of the capsule was opened; this led to a decrease in oxygen content to 27.5 percent. In the following days its content increased steadily and reached 69 percent at the end of the 8th day. From that time onwards until the beginning of the 12th day the oxygen content fluctuated between 69 and 64 percent and then decreased slowly until it dropped to 18.5 percent at the end of the 14th day. The increase in air changes in the regenerating system from 10 to 16 liters per minute also did not produce positive results. With the increased ventilation the percentage content of oxygen increased rapidly and then decreased equally rapidly in spite of the fact that the rate of ventilation was maintained.

The carbon dioxide content fluctuated throughout the whole experiment between 0.1 and 0.4 percent and only in certain cases was an increase of up to 0.6-0.7 percent observed. Air temperature in the pressurized capsule was 18-24$^{\circ}$ C, which was only 2-3$^{\circ}$ C higher than the ambient air temperature.

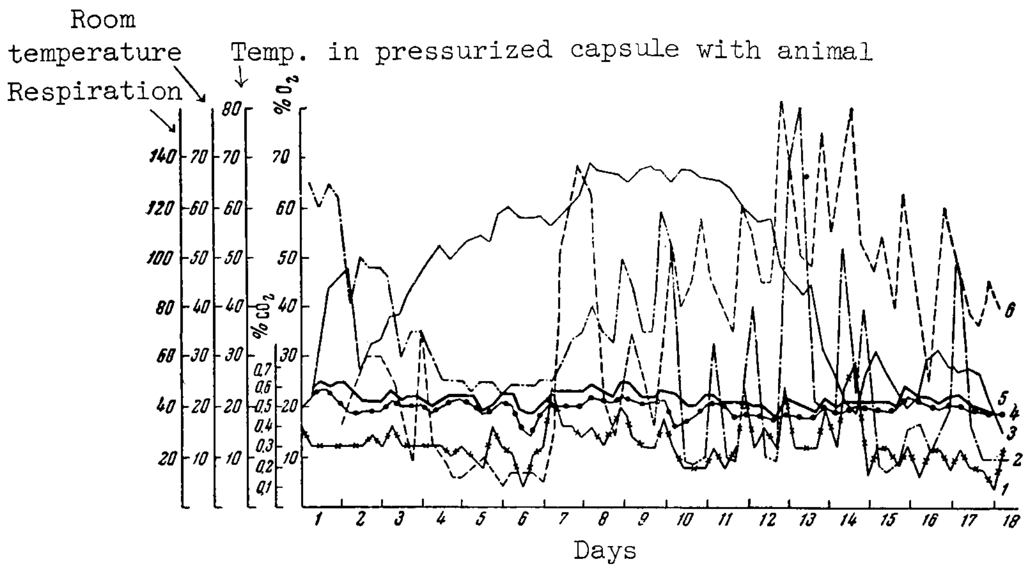


Figure 2. Graph Showing Variations in Hygienic Parameters of the Medium in the Pressurized Cabin and the Respiration Rate of the Animal During the Experiment of the First Series

(Dog, Umnitsa, March 27, 1957): 1 - % $CO_2$; 2 - Respiration Rate; 3 - % $O_2$; 4 - Room Temperature; 5 - Capsule Temperature; 6 - % Relative Humidity

The relative humidity of the air gradually decreased from 65 percent at the beginning of the experiment to 25 percent, and remained at this level to the end of the 7th day. Thereupon it gradually increased. The graph shows that the relative humidity curve had a "sawtooth" character; it increased to 50-60 percent during the day and dropped to 35-40 percent during the night. From the 12th until the 14th day of the experiment the diurnal fluctuations of relative humidity were very noticeable: at night 48-55 percent and during the day - 80 percent. The increased water vapor concentration in the air of the capsule during the day was most likely due to the more active behavior of the animal and also due to the issuing of food and water. From the nature of the relative humidity curve it is possible to estimate, to a known degree, the stability in the performance of the regenerating system. In the above experiment this was particularly well revealed between the 12th and 14th days when considerable fluctuations in the relative humidity preceded a rapid fall of oxygen concentration in the air of the capsule.

Considering the complexity of controlling the regenerating system during the flight of an artificial Earth satellite, in the laboratory

experiments particular attention was paid to automation and to insure trouble-free operation. The main criterion used in assessing the system was the stability in maintaining the necessary living conditions during a given period of time without any interference from the experimentor. Starting with these assumptions, the normal service life of the system in the above experiment should be considered to be 14 days, after which time oxygen concentration in the atmosphere of the capsule decreased to 18.5 percent. A further extension of the duration of the experiment could only be achieved by increasing the throughput of the air processed in the installation. Analysis of the regenerating substance for oxygen, carbon dioxide and moisture after the experiment showed that the substance in the units was getting exhausted unevenly and contained a considerable quantity of oxygen. In the front part of the regenerating installation (i.e. in the first unit from the air inlet) the substance was exhausted sufficiently fully and evolved almost all of the oxygen stored. However, in the rear part, i.e. in the second unit (at the outlet of the installation), a large part of the substance remained unused and contained more than 650 liters of oxygen. Similar results were obtained in numerous other experiments.

The incomplete exhaustion of the reagent was found to be due to the uneven rate of air flow in the different sections of the installation. In the first unit the air flowed uniformly through all the substance with the same velocity across the whole cross-section of the duct. In the semi-toroidal joining tube, which did not contain any reagent, the uniformity of air flow was considerably disturbed. As a result, the movement of the main mass of air in the second unit was directed along the walls. The substance which was placed in the central part of the unit did not have sufficient contact with air and consequently remained unexhausted. The chemical nature of the reagent was also responsible to a certain extent for the incomplete and uneven exhaustion of the substance which, depending on the temperature and absolute humidity of the regenerated air, may absorb carbon dioxide in quantities exceeding considerably its available supplies of oxygen. In the given case, the substance placed in the first unit at the air inlet may evolve all the oxygen and still partly absorb water vapor and carbon dioxide, impeding the active work of the substance in the second unit.

In this way, the ventilating system in the first series of experiments did not give a uniform and complete exhaustion of all the available supplies of the substance. An increase of the air throughput to two or three times the calculated value (e.g., in experiments 121, 122) did not increase utilization of the reagent but led to a considerable increase of the percentage content of oxygen in the air of the cabin.

In the subsequent stage of the investigations it was decided to change radically the path of the airstream. The changes involved making both fans work as expellers pumping out the air which was entering through

an opening in the middle of the rear wall of the tube joining the regenerating units (see Figure 1b middle). It was assumed that the air from the cabin would pass through the regenerating substance of both units with uniform and equal velocities and, after becoming enriched with oxygen, would be supplied for breathing to the animal.

It was found experimentally that the regenerating substance evolved oxygen and absorbed carbon dioxide and moisture at a higher rate and more uniformly than in the experiments of the earlier series. However, optimal conditions with regard to oxygen content were attained only in isolated experiments. In the majority of experiments oxygen concentration in the cabin became so high within the first few days that it could be harmful to the animal.

Experiment 128, of approximately 19 days' duration, was found to be completely satisfactory; all the required parameters of the microclimate of the cabin were retained throughout the whole duration of the experiment (Figure 3).

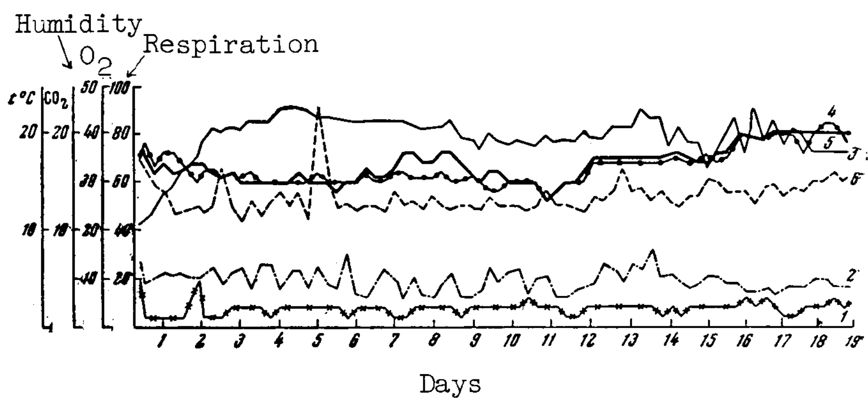


Figure 3. Graph Showing Variations in Hygienic Parameters of the Medium in the Pressurized Capsule and the Animal's Respiration Rate during the Experiment of the Second Series

(Dog, Layka, Experiment 128): 1 - % $CO_2$; 2 - Respiration Rate; 3 - % $O_2$; 4 - Room Temperature; 5 - Capsule Temperature; 6 - % Relative Humidity

The oxygen content increased rapidly at the beginning of the experiment, reaching 45.3 percent at the end of the 4th day. From then onwards this concentration became more stable; a decreasing tendency bringing it down to 36-40 percent was observed between the 9th and 12th days, while from the 13th day until the end of the experiment it fluctuated between 36 and

45 percent. The carbon dioxide content did not exceed 0.2 percent during this experiment and only in isolated cases did it reach 0.3-0.5 percent. The relative humidity was within 22-35 percent, and the temperature within 14-20° C.

In contrast to the above, in the experiment started on August 20, 1958 (Figure 4), already at the beginning of the 3rd day the oxygen concentration reached 60 percent and on the 5th day it was approaching 80 percent. The maximum carbon dioxide concentration hardly reached 0.4 percent; the relative humidity from the 6th-7th day onwards until the end of the experiment reached, with certain fluctuations, 80 percent and more. It is clear that from the point of view of gas composition, such conditions cannot be considered as satisfactory and completely harmless to organism.

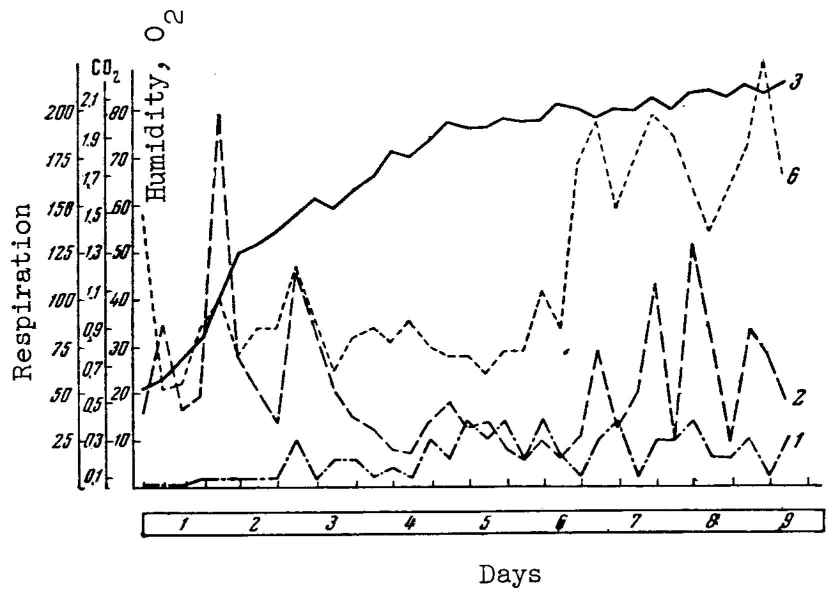


Figure 4. Graph Showing Variations in Hygienic Parameters of the Medium in the Pressurized Capsule and the Animal's Respiration Rate during the Experiment of the Second Series

(Experiment Starting on August 20, 1956): 1 - % $CO_2$; 2 - Respiration Rate; 3 - % $O_2$; 4 - Room Temperature; 5 - Capsule Temperature; 6 - % Relative Humidity

The results of the second series of experiments, although better than the first series, showed nevertheless that simultaneous introduction

into the system of the whole supply of reagent does not secure sufficiently uniform utilization and in most cases leads to a high percentage of oxygen in the air of the cabin.

In order to secure uniform utilization of all the substance it would be necessary to place it in separate small sections with 2-3 days' oxygen supply each. As the substance becomes exhausted in one section, the ventilating system would be automatically switched over to the next section. The control of the timing mechanism could be achieved by a clockwork mechanism or by means of pressure in the cabin - a sensitive aneroid could be used for the latter. The above conclusions were reached on the basis of all the experimental results. The suggested method would insure proper utilization of the substance and thus provide for the animal optimal conditions with regard to air composition.

The main difficulty of applying the different variants of the above described system under actual satellite flight conditions is due to size limitations, which do not permit placing the regenerative substance in separate small sections.

Switching of ventilation from one section to the other also involves a complex automatic system, which in view of its dimensions, weight and the limited sources of power supply, could not be adopted in practice.

In the third series of the experiments, the substance was made to work in units actuated by the automatic control mechanisms of the ventilation in the regenerative system. For this purpose a controlling aneroid was connected in series into the electrical circuit supplying the motor of the ventilator. When the pressure in the cabin fell below 765 mm Hg the circuit was made and when this figure was exceeded it was broken (see Figure 1b right). In this way the substance of the first unit of the installation was working continuously while that of the second was switched on only when the pressure in the cabin fell to or below 765 mm Hg.

Data obtained during extensive experiments showed that the last mentioned system of ventilation in the regenerating installation works faultlessly and is stable. The on and off switching of the ventilator motor proceeds smoothly and accurately in accordance with the predetermined pressure. Tests carried out under conditions of overload and vibration also gave good results.

Consequently, by increasing the barometric pressure in the cabin above the predetermined level (765 mm Hg), the increased rate of oxygen production effects the switching off of one of the regenerating units and conversely when the pressure falls the unit is switched on again. The examples quoted illustrate the hygienic conditions established in the cabin during certain tests of the third series (Figures 5 and 6).

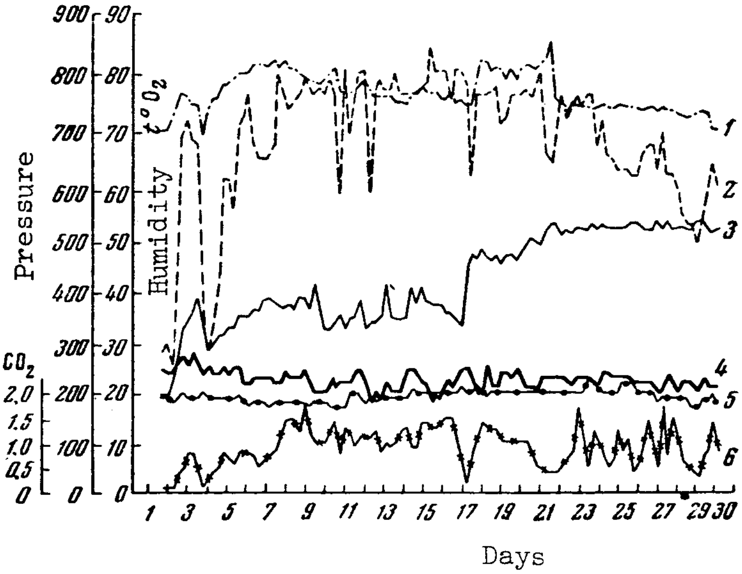


Figure 5. Graph Showing Changes in the Hygienic Parameters of the Medium in the Pressurized Capsule during Experiments of the Third Series

(Dog Umnitsa, Experiment 134): 1 - Pressure; 2 - Relative Humidity (%); 3 - Oxygen Content (%); 4 - Temperature in the Capsule; 5 - Room Temperature; 6 - $CO_2$ Content (%)

In experiment 134 (Figure 5) oxygen concentration during the first 16 days was within 30-40 percent, and from the 17th day until the end of the test it was 50-55 percent. Carbon dioxide concentration fluctuated from 1.0 to 1.5 percent, reaching in isolated cases 2 percent. Relative humidity of air in the cabin changed considerably during the experiment. Appreciable fluctuations in the level of $CO_2$, $O_2$, relative humidity and

barometric pressure in the cabin were produced by periodic switching on and off of one of the ventilators as well as by repeated stoppage of the motor due to technical reasons, followed by its re-starting.

Once, in the above described experiment (of February 24) the excess pressure in the cabin was released to the Douglas bag for technical reasons. All this changed somewhat the picture, on the basis of which one could assess the performance of the third variant of the regenerating plant. However, in general the experimental and other supporting evidence suggests that this variant is suitable for the regenerating plant and its self-regulating system.

During experiment 133 (Figure 6) the oxygen concentration in the cabin increased only slowly, remaining within 20-30 percent during the

whole experiment. There was a correspondingly small change in the barometric pressure. $CO_2$ concentration stayed at approximately 1 percent, with a maximum of 1.5 percent and a minimum of 0.5 percent. Variations of equally small magnitude were observed in relative humidity, which remained at 75 percent. Hence the hygienic parameters in experiment 133 were sufficiently stable to satisfy the requirements.

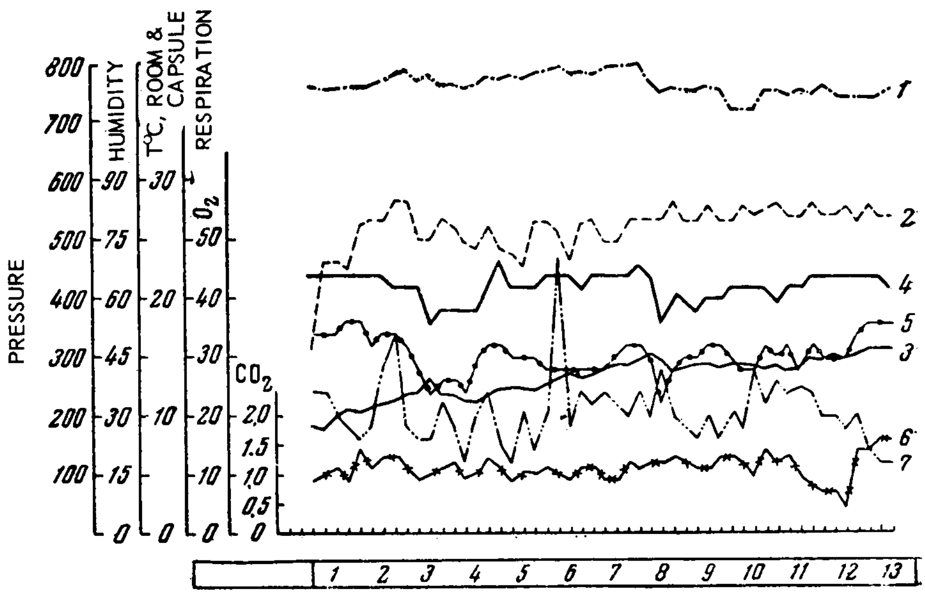


Figure 6. Graph Showing Variations in Hygienic Parameters of the Medium in Pressurized Cabin and Animal's Respiration Rate in the Experiment of the Third Series

(Dog Zhuchka, Experiment 133): 1 - Pressure; 2 - Relative Humidity (%); 3 - Oxygen Content (%); 4 - Temperature in the Capsule; 5 - Room Temperature; 6 - $CO_2$ Content; 7 - Respiration Rate of the Animal

Thus, we have described the path followed in developing the optimal method of air regeneration satisfying the specific conditions of the experiment. Highly active chemical reagents were found to most suitable which, in addition to moisture and $CO_2$ absorption evolve the necessary quantity of oxygen. The air entered the regenerating plant through the opening in the central part of the channel joining both units and was passed separately into each unit. The airflow was produced by motor driven ventilators. Automatic adjustment of the regenerating plant was

achieved by means of a special aneroid incorporated in the electric circuit which switched on and off one of the regenerating units. This arrangement provided adequate stability in the optimal medium parameters in the cabin, viz, barometric pressure, atmosphere composition and humidity.

The above described method was successfully employed in the experiment with the dog Layka in the second Soviet artificial earth satellite.

Summary

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A system has been developed for regenerating the air in sealed capsules occupied by animals during space flights. A chemical method was used (Important note: the actual compounds used are not specified) and the air was brought in contact with the regenerator by electric fans. The total weight of the installation was 1/8 of that of a conventional system using soda-lime, silica gel and oxygen gas. The air in sealed containers occupied by dogs weighing 5-7 kg could be maintained in a satisfactory condition by means of the installation for periods as long as 30 days and the system was subsequently used in the second space flight with the dog, Layka.

Belkon

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# PREPARATION OF THE ANIMAL PRIOR TO THE EXPERIMENT

O. G. Gazenko and V. S. Georgiyevskiy

The preparation of the biological studies on artificial Earth satellites included the solution of a number of methodological problems, such as choice of the animal and development of a definite system for its preparation and training prior to the experiment. When solving the above problems the conditions of the experiments were taken into consideration, namely that the experimental animal would have to remain for a long time in a small pressurized cabin, receive its food from an automatic dispenser and be exposed to accelerations, vibrations, noise, fluctuations in barometric pressure and other factors.

For that reason it was necessary to select relatively small animals, resistant to the effects of unusual external factors and capable of acclimatizing themselves to prolonged stay in a confined space.

It was particularly desirable to utilize in the experiment higher monkeys which are philogenetically closest to human beings. However, these animals are very difficult to train under experimental conditions. The use of small lower monkeys would not give any particular advantages as compared with other laboratory animals and, from the methodological point of view, would be far more complicated. Small laboratory rodents (rabbits, rats and mice) were very difficult from the point of view of attaching various sensors registering physiological functions. In addition, small animals were characterized by a very high frequency of cardiac contractions and high respiration rates. For instance, under certain conditions the frequency of cardiac contractions in mice may reach up to 600 per minute (Zhedenev, 1954). In order to obtain a satisfactory registration at such frequencies, a very high scanning velocity is required involving considerable expenditure of materials, which is not desirable when carrying out prolonged investigations.

At the same time small laboratory rodents were found to be very useful for the purpose of explaining a number of special problems, for instance, the effect of cosmic radiation, coordination of movements in the condition of weightlessness, etc. (Simons, 1954; Chase, 1952; Henry et al., 1952).

Dogs could also be used as suitable experimental objects (Pavlov, 1951). The normal physiology of these traditional laboratory animals has been well studied. The control of the main physiological functions (respiration and blood circulation), in contrast to a number of other animals, is the same in dogs as in humans. Dogs submit easily to training

and rapidly become accustomed to a stay in a confined capsule, isolated from external stimuli. They also behave very calmly while immobilized. As regards the organization and functional peculiarities of the nervous system, dogs are on a sufficiently high level of development to make them suitable subjects for the purpose of physiological investigations (Voronin, 1959; Koshtoyants, 1957).

A further problem arises in the selection of the dogs most suitable for the experiments which are based on a number of particular requirements.

The size of the dog was limited by the volume of the capsule assigned for the accommodation of the animal (600 x 220 x 450 mm), consequently, it was necessary to use only relatively small animals weighing 5-6 kg.

Since the age of an animal brings about certain changes in the characteristic functions and the structure of the organism that have a direct bearing on the resistance of the animal during exposure to various factors, it was considered desirable to use dogs from 1.5-2 to 5-6 years old.

It is known from laboratory practice that thoroughbred animals are seldom resistant to the unfavorable environmental effects and for that reason preference was given to low-bred dogs - plain mongrels (Speranskaya, 1948).

When designing the sanitary devices it was found that simpler and safer urine and faeces receptacles could be provided for the bitches than for dogs. For this reason bitches were selected for the experiments. An animal's hair is continuously being shed; it would thus accumulate in the capsule and this could interfere with the instruments and the regenerating plant. Therefore, it was necessary to select dogs with short hair. Although it was known that short-haired dogs were less resistant to temperature variations, this was not considered a serious objection, since in the capsule the animal was dressed in rubber and fabric clothes, which created particular conditions of heat exchange and gave an equal chance to all the animals (Slonim, 1952).

General considerations and the laboratory results showed that the ideal objects for such experiments are dogs of the so-called restrained type (Pavlov, 1951). Animals with this type of nervous system submit well to training for the experimental requirements and it is easier to suppress in them the so-called "freedom reflex" (Pavlov, 1951).

The successful use of dogs in experiments carried out on research rockets made it possible to expect that they would also be suitable for the satellite experiments. However, the considerably longer duration of the latter experiments required the development of a far more complex system of preparation and training. The experimental conditions featured

a considerably greater number of external variables. For that reason the dogs were in turn accustomed to prolonged stay in a small closed capsule with considerably restricted movement, to reception of food and water from automatic devices, to the wearing of sanitary and immobilizing apparel, as well as sensors, etc. In addition, it was necessary to suppress, as far as possible, the orientating and defensive reactions of the animal to all kinds of unexpected external stimuli, such as the adjustment and actuation of the apparatus, variations of pressure or temperature of the air, etc. A separate problem was presented by the training of the animals to endure the effects of certain flight factors (acceleration, noise, vibration) but this aspect is treated in greater detail in special papers included in the present symposium.

In the course of training, the dogs were successively and gradually accustomed to the action of an increasing number of environmental conditions. After selection and careful preliminary inspection, the animals were trained for laboratory conditions and put into special cages. The size of these cages was gradually decreased, approaching the dimensions of the space available for the dog in the pressurized capsule of the satellite. This stage of the training was completed by placing the dog in a cage 540 x 410 x 200 mm (Figure 1).

Figure 1. Dog in a Small-size Training Cage. The Side Panel is Lifted Off.

Although the experiment on the satellite was envisaged to last 7 days, it was considered expedient during the training to extend the residence of the animals in cases and capsules to 20 days. The length of stay of the dogs in a restricted space was increased from a few hours to a final stay of 15 to 20 days.

The animals were simultaneously accustomed to wearing the sanitary and immobilizing apparel. Here, the defecation and urination presented some difficulties. In the case of insufficiently trained dogs, prolonged immobilization and application of the above mentioned apparel caused at first some constipation and ischuria, which in turn led to some motor disturbance and occasional upset of general well-being. Application of drugs (various laxatives) failed to give a stable effect. Only stage by stage and consistent training insured normal functioning of the organism.

The first stage of training was considered completed when the dogs endured calmly a 20-day stay in a confined cage with all the equipment without exhibiting any disorders or any local injuries. In the following stage of training the dogs were accustomed to a long stay in a pressurized capsule. A model of a pressurized capsule of the satellite (diameter 640, length 800 mm) was provided which carried all the necessary equipment for the future flight (Figure 2). The dog became acquainted with the surroundings of the capsule, to the receiving of food from the automatic dispensers and to the noise of the equipment assemblies. Gradually the reactions of the animal to the complex set of stimuli due to the assembly of the apparatus and pressurization were suppressed. The stay of the animals in the pressurized capsule was steadily increased from 3 to 20 days.

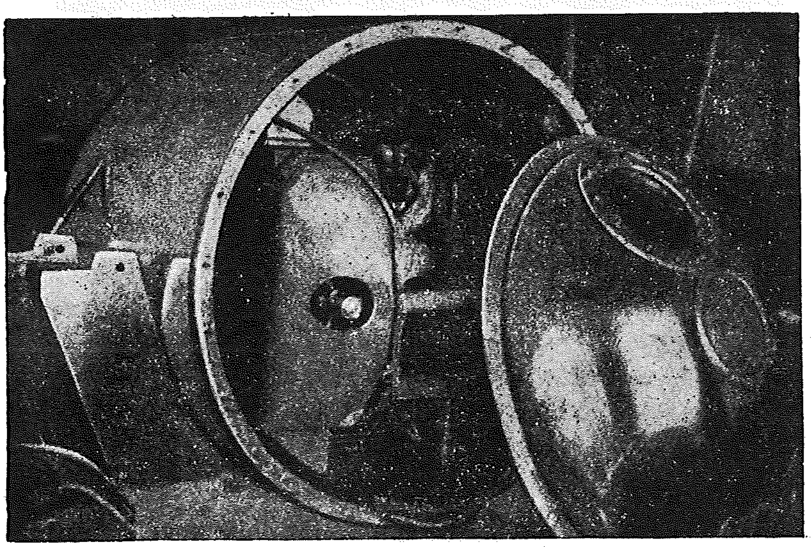

Figure 2. The Dog Umnitsa in a Model of a Pressurized Capsule of a Satellite. The Front Cover is Removed.

During these experiments observations on the general state and behavior of the dogs were carried out and the frequency of breathing and pulse rate registered.

In order to estimate the motor activity of the animals, the readings of the movement-sensor recorded on a barospeedograph K-2-74 were used. Before and after each training experiment, the animals were carefully examined, temperature taken, weighed, blood analyzed and chest X-rayed.

Out of ten dogs who completed the first stage of training in confined cages, six were selected for tests in a pressurized capsule. Altogether 27 training experiments lasting from 6 to 20 days each, were carried out.

For example, during the training of Layka (experiment 119, of 19.5 days duration), the behavior of the animal was calm with the exception of the first two days and an increased motor disturbance during the 14th day when the concentration of carbon dioxide rapidly increased as a result of a stoppage of the ventilator motors in the regenerating plant. Only during the first two days did the frequency of respiration reach 60 per minute, and then for the whole remaining period of the experiment it was within 20-35 respiratory movements per minute.

Upon completion of the experiment no disturbances in the general state and behavior of the animal were found with the exception of a moderate loss in weight.

Analysis of the data obtained during the training tests has shown that the animals endured satisfactorily a period of up to 20 days confinement in a pressurized capsule during which time their reactions from the cardiovascular and respiratory systems were within the normal fluctuations.

At the same time, in a number of tests, during which the concentration of oxygen or carbon dioxide in the chamber did not correspond to the preset quantities, or when the predetermined temperature conditions were not maintained, definite changes were observed in the behavior and physiology of the dogs. This circumstance made it necessary to carry out separate experiments which would make possible accurate evaluation of the permissible fluctuations in the above parameters of the ambient medium. Main attention was paid to determination of the upper limit of the permissible concentration of oxygen to which an animal could be exposed during a prolonged stay in a capsule and to the permissible increase of temperature in the small capsule with the dog in it. The data obtained were used to confirm the requirements necessary for the conditioning and regeneration of the air in the pressurized capsule of the satellite.

The preparatory period included operating on the animals for methodological purposes. To make possible the registration of the arterial pressure, all the experimental animals were operated upon prior to the experiments in order to insert the carotid artery into a skin flap. In addition, an operation to graft electrodes for the registration of electrocardiogram was also carried out. Silver electrodes in the shape of small rings were sewn with silk to the rear mid-axillary line to the fasciae of the muscles, from both sides, in the region of the 4th-5th intercostal. The wires of the conductors were taken out on both sides of the backbone at a distance of 15-20 mm from each other and at the level of the first pectoral vertebra. During the preparation, the animals were offered food of the type generally given in the vivariums of the physiological laboratories (Kogan, Shchitov, 1954). During the training in the pressurized capsule, the dogs received once a day a special food ration from the automatic dispensers.

The information obtained during the preparation of the animals for the experiment on the satellite, showed that trained dogs could endure satisfactorily prolonged (up to 20 days) stays in a pressurized capsule. This procedure made it possible to start direct preparation of the animals for the satellite experiment.

Of all the animals which were subjected to the full training program, the dog Layka was chosen, who was a short-haired bitch about 2 years old and weighing 6 kg.

Summary

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Dogs are considered to be the most suitable animals for space flight experiments; they should be crossbred males, not more than 6 kg in weight and 1.5 to 6 years old. The animals are first trained to become accustomed to confinement for 20 days in cages of gradually decreasing size, the final one being 54 x 41 x 20 cm. They are then adapted to existence in a hermetically sealed container 64 cm in diameter and 80 cm long containing all the appropriate equipment which is required for the space flight. The described procedure was used in selecting the dog Layka for the successful space flight.

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# SANITARY DEVICES IN PRESSURIZED CAPSULES

O. G. Gazenko, A. A. Gyurdzhian and G. A. Zakhar'yev

When an experimental animal is isolated for several days in the confined space of the pressurized capsule of an artificial Earth satellite, the necessity arises for the development of a special sanitary system. The methods of sanitation employed during prolonged experiments in an isolated capsule are of considerable interest for various other experimental investigations; for example, metabolism studies. In a number of cases, a separate collection of urine and faeces at the same time is also desirable.

The development of these sanitary devices consisting of special urine and faeces receptacles is a particularly difficult problem. The main difficulty lies in being able to fasten the receptacles securely to the animal throughout the whole of the experiment, without disturbing or causing any superficial damage to the dog, irrespective of its motor activity. At the same time, the sanitary device should permit sufficient freedom of movement necessary for the continuance of the experimental animal's normal vital activities.

The problem is further complicated by the nature of the experiments in the isolated pressurized capsule of an artificial satellite which excludes the possibility of introducing during the course of the experiment any modifications necessary for the correction of any errors that may arise. Information about the conditions of the ambient medium, the position and general condition of the dog in the capsule are received by the experimeter only by means of sensors fixed to the animal, and mounted in the capsule. All of these factors impose special requirements which limit the design of the sanitary devices, and the materials and methods used in their manufacture. Satisfactory results in this branch of space technology were obtained only after prolonged testing of many modifications of the sanitary devices in numerous experiments, often of 20 days duration.

In the literature are described methods for obtaining and collecting excreta from experimental dogs which had been previously prepared by the appropriate operations in which their ureters were exposed and various fistulae created. Methods for the collection of urine on its own in metallic containers strapped to the body of a dog have also been reported. These receptacles however, can only be used in short-term experiments, as they are not very securely fastened to the animal and can cause a significant amount of skin irritation as well as restrain its freedom of movement, and so produce acute disturbance in the animal (Haberland, 1934).

Because of the anatomical features of the excretory system of bitches and dogs, two different variants of the sanitary device had to be made: ASU-1 for bitches and ASU-2 for dogs, which differed in the design of the urine and faeces receptacle. The modifications of the devices developed consist of a special urine-cum-faeces receptacle adhering closely to the pelvic region and a special "brassiere" on the shoulder girdle for attaching the receptacle to the dog (Figures 1-5). The different parts of the ASU were prepared from a rubberized knitted fabric, vulcanized rubber, soft spongy rubber and, in one of the variants (ASU-2), steel wire. The combination of rubber and fabric insured the required elasticity and strength of the equipment. The rubber used in these articles does not contain any odorous or skin irritating substances and also fulfills a number of the physico-mechanical and chemical requirements.

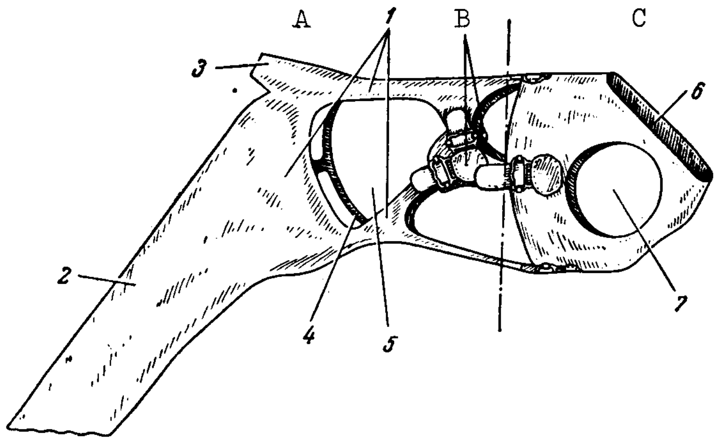


Figure 1. Diagram of the Sanitary Device ASU-1

A - Receptacle for Urine and Faeces; B - "Brassiere"; C - Straps; 1 - Shorts; 2 - Duct; 3 - Opening for the Tail; 4 - Obturator; 5 - Opening for the Hind Legs; 6 - Opening for the Head; 7 - Opening for the Front Paws

The complete sanitary device covers a relatively small surface of the dog's body and does not interfere with its normal heat loss and hence the control of its body temperature. Actinometric determinations have shown that the portions of the body covered by the sanitary device radiate the same amount of heat as those left uncovered.

The sanitary device for bitches (ASU-1) was used during the experiment with Layka in the second artificial Earth satellite, and also on the second and subsequent cosmic ship-satellites. The "brassiere" of the device was made from a rubberized knitted material (fabric T-1, and rubber

B-201) and has two openings for the front paws and one large opening for the head. The edge(s) of the latter are fastened by means of a fabric strip glued along the perimeter (Figures 1, 2). (Translator's note: It is not clear from the Russian test whether "latter" refers to one or all the three openings.) The urine and faeces receptacle (ASU-1) consists of the following parts:

1. Shorts of rubberized knitted fabric, together with straps for the attachment of the urine and faeces receptacle to the "brassiere". The shorts encircle the body of the dog from the top to the bottom of the pelvic region. To the shorts is attached (glued) a special obturator with a pipe (Figures 1, 2, 3).

2. An obturator made of a soft, spongy rubber which has an oval opening facilitating the passage of the faeces and urine, the whole fitment corresponding in dimensions and design to the part of the body which it fits. The oval opening of the obturator is provided with a crosspiece which is placed on the isthmus between the anus and the external genitalia. The tail of the dog passes through the upper half of the obturator's opening. So as to avoid lesions of the internal surface of the hips and the inguinal region, the rim and the opening of the obturator narrows as it passes backwards and downwards. The obturator is slightly bent to conform with the shape of the body.

3. A duct from the urine and faeces receptacle in the form of a long, flexible tube turns slightly downwards and drains the faeces and urine from the receptacle.

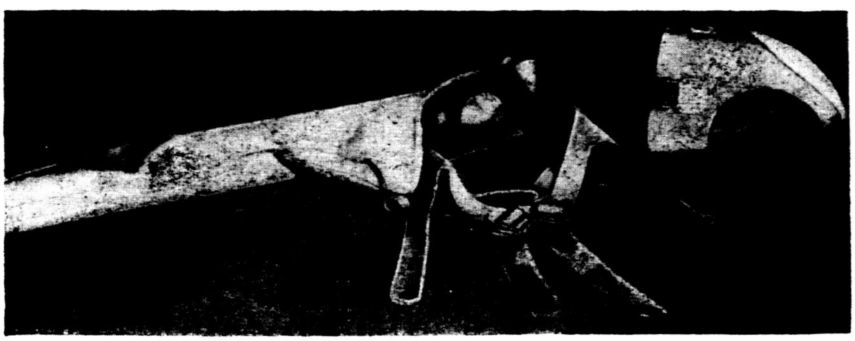

Figure 2. Sanitary Device ASU-1

The duct is made of vulcanized rubber (B-201). It is bent downwards and runs to a metallic tube in the top of the sanitary tank which lies beneath the floor of the pressurized cabin. In the floor, somewhat towards the rear of the hind legs of the dog, is an opening (60-70 mm diameter) through which the duct passes freely without any additional fastening.

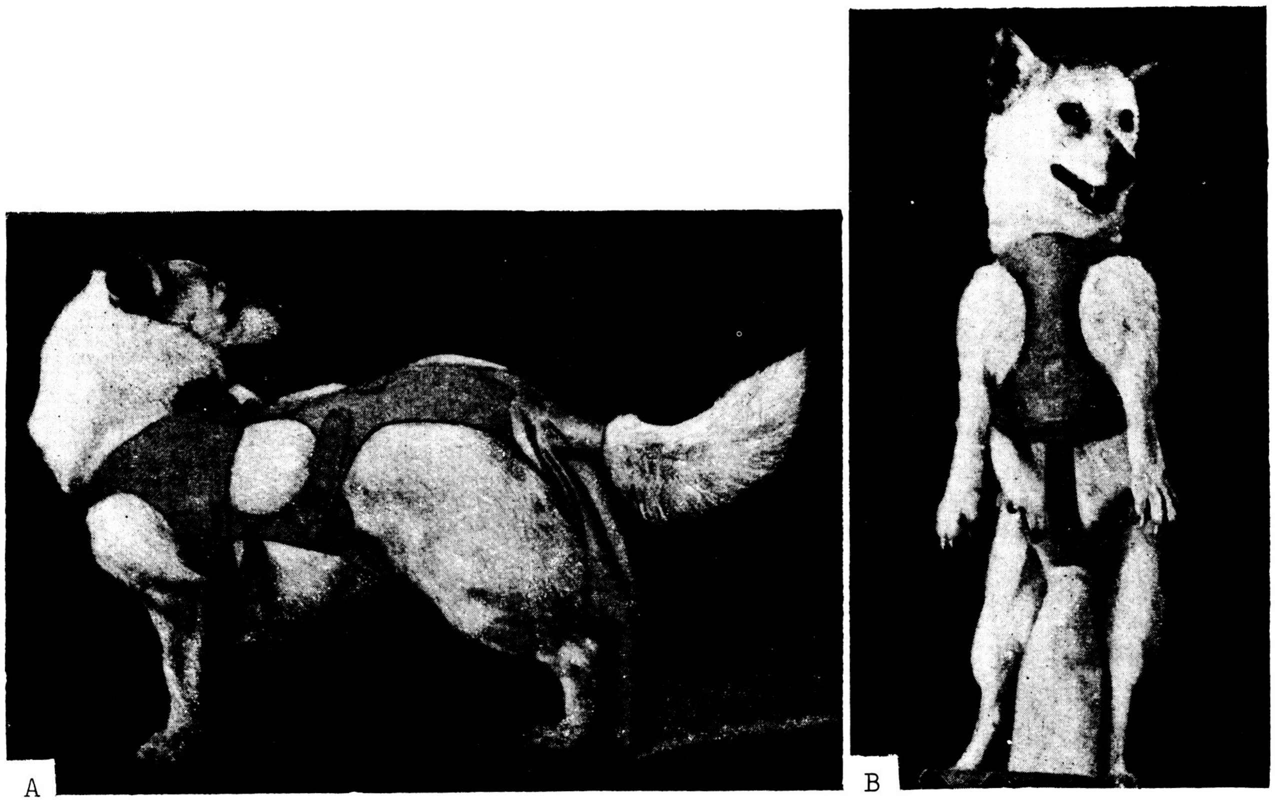

Figure 3. A Dog Carrying Its Sanitary Device

A - Side View; B - Front View

The length of the duct permits the dog to get up, lie down and accomplish certain other movements. On the upper part of the rear wall of the duct is an opening with a rubber sleeve shaped like a truncated cone through which passes the tail of the dog. The sleeve is made of fine rubber and surrounds the tail without impeding its blood flow or causing any other lesions. The opening with the rubber sleeve through which the tail passes serves as an important additional point for attaching the urine and faeces receptacle to the dog's body. Inside the duct and along its entire length (i.e., from the crosspiece of the obturator's opening to the sanitary tank), a partition can be inserted for separating the urine from the faeces. As the experiments so far carried out did not require the separate collection of urine and faeces, the partition was not inserted in the duct.

The "brassiere" and the urine faeces receptacle are connected by means of the above-mentioned adjustable straps which have self-tightening buckles. The external surface of the "brassiere" carries four buckles which are not in contact with the body of the dog. Six straps run from the shorts of the urine and faeces receptacle (three from the top and three from the bottom) also, separately, two ties (rings) each of which carries a strap and two buckles. The upper and the lower straps are fastened along the mid-line of the body with the corresponding buckles of the "brassiere". The lateral straps are connected in pairs (the upper and lower on each side) by means of ties (rings) and a single strap with the lateral buckles of the "brassiere". The ties (rings) permit the exact adjustment of the length of the straps and so enable the urine and faeces receptacle to be attached securely to the body of the dog. The free ends of the straps are glued with a rubber solution to the underlying surface of the sanitary device so as to prevent the buckles loosening. Before the urine and faeces receptacle is fastened onto the dog, a liberal layer of penicillin ointment is applied to the perineum and inguinal regions so as to protect these surfaces from irritation and maceration by the urine and faeces. Deodorants and hygroscopic substances (dried moss and activated carbon) are placed into the metallic tank of the sanitary reservoir (Figure 4) which helps to fill the reservoir uniformly with the liquid part of the excreta. The capacity of the tank is 6-8 liters. Bitches weighing 4-7.5 kg were used in the experiments.

To match the dimensions of the sanitary devices with the dimensions of the dogs, they were made in three standard sizes, the smallest (1), medium (2) and outsize (3). The size (3) sanitary device was used in the experiment with Layka. To determine the size of the sanitary device to be used, the measurements necessary are made directly on the experimental dogs concerned. The anatomical peculiarities of males complicated the design of the sanitary device, in particular the construction of the urine and faeces receptacle. It was found necessary to make separate obturators for the collection of the urine and faeces. The main difficulty lay in making the crosspiece placed between the anus and the penis. It should

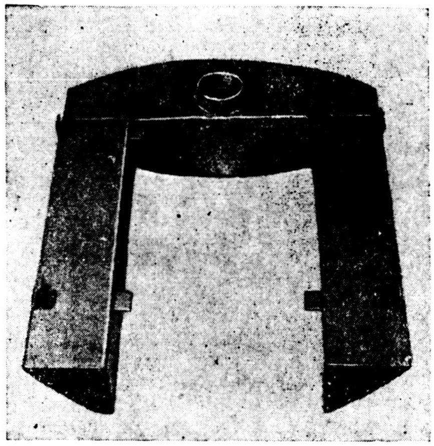

Figure 4. Sanitary Tank

not seriously affect the design of the device and so interfere with its attachment to the body of the animal. At the same time it should pass above the scrotum without injuring it and without causing any skin irritation. After testing many types of device, the sanitary device ASU-2 for males was made which fulfilled all the above requirements (Figures 5, 6).

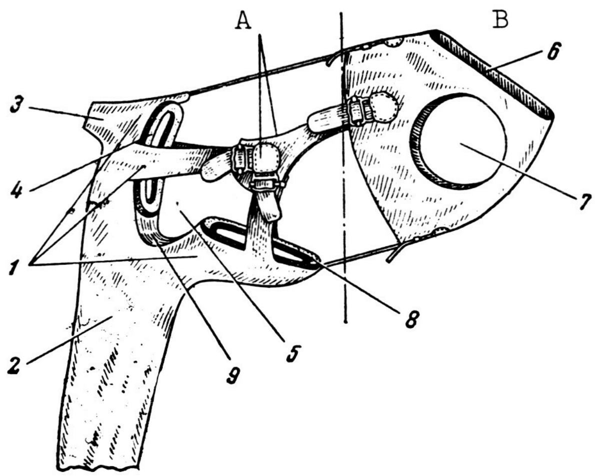


Figure 5. Diagram of the Sanitary Device ASU-2

A - Urine and Faeces Receptacle; B - "Brassiere"; C - Buckles; 1 - Shorts; 2 - Duct; 3 - Opening for Tail; 4 - Obturator for Faeces; 5 - Opening for Hind Legs; 6 - Opening for Head; 7 - Opening for Front Paws; 8 - Obturator for Urine; 9 - Crosspiece

The urine and faeces receptacle ASU-2 has two obturators made of soft, spongy rubber which are oval in shape and adhere tightly to the body of the dog. The upper obturator surrounds the anus and the base of the tail. The lower adheres to the pudendal region and surrounds the penis, its rear part lying between the base of the corpora cavernosa and the scrotum.

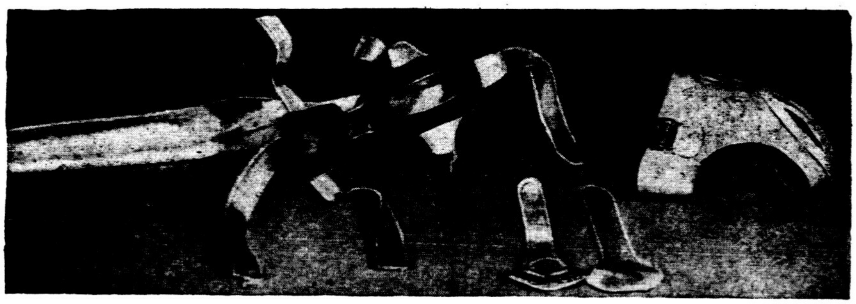

Figure 6. Sanitary Device ASU-2

The obturators are connected by a crosspiece of 22-25 mm in width and approximately 80 mm long. A steel wire, the ends of which surround the ring of the obturators, serves as the frame. The crosspiece is covered on its outer surface with rubber while the surface facing the body of the dog is lined with chamois leather. Due to the elasticity of the crosspiece, it may be bent and given any form - corresponding to the configuration and individual dimensions of the underlying part of the body. The crosspiece passes over the surface of the body without touching it and without irritating it. By changing the shape of the crosspiece it is also possible to adjust the distance between the obturators which lead into the single duct. The ASU-2 also differs slightly in other respects from the ASU-1, namely, the shape of the shorts and the place where the adjustable straps are fastened, both of which can be clearly seen in the figures.

Systematic training of the animal, so that it becomes accustomed to urinating and defecating regularly while wearing the sanitary device and other pieces of harness in the pressurized cabin, is an important part of the preparation for these experiments. In order to avoid reflex suppression of defecation, constipation, and ischuria, which induce restlessness in the dog, it is necessary to carry out stage-by-stage training for accustoming the animal to the conditions of the restricted space of the capsule, its relative immobility, and the wearing of the sanitary device and other fabric apparel. Sometimes it is necessary to resort to small doses of laxatives (castor oil, isaphenin, purgene, etc.). However, in most cases the program can be completed without resorting to pharmaceutical aids.

The experiments have shown that when the above conditions are fulfilled, sanitary devices can be fully utilized during a 20-day test, the excreta falling into the tank via the duct. The dogs endured the experiment very satisfactorily, and with properly adjusted sanitary devices and harness they suffered no injuries to the skin or other pathological changes. The behavior of the dogs was calm.

The average daily weight of the excreta of the dogs in the above experiments did not exceed 200 g. However, this data should be taken as only tentative when calculating the capacity of the sanitary reservoirs, as there may well be differences due to the specific food ration of the animals, the dog's weight and other individual variations.

Because of hygienic considerations, a sanitary device should be used only once in a long experiment.

Summary

11693

A device to collect and remove the excreta of animals that remain in a sealed cabin for many days is described. The device is made of rubber-impregnated knitted fabric and worn by the animal. The excreta are collected in a special can.

The device functions quite well, and gives the animal sufficient freedom of movement and does not annoy it during the 20-day experiment. The technique described was successfully used for Layka on the second earth satellite and on the subsequent satellite spaceships. Author

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# FASTENING OF AN ANIMAL IN A PRESSURIZED CAPSULE, FABRIC APPAREL AND DISTRIBUTION OF SENSORS FOR THE REGISTRATION OF PHYSIOLOGICAL FUNCTIONS

O. G. Gazenko and A. A. Gyurdzhian

To carry out a long experiment on a dog kept in a pressurized capsule of an artificial satellite which is of limited size, certain specific requirements must be met with regard to fastening the animal in the capsule, and also to the disposition and methods of fastening the sensors which register any of the changes that may occur in the animal's physiology. The fastening should retain its correct position on the body and so prevent any damage occurring to the leads running to the sensors, or to the sensors themselves. At the same time, the fastening devices must not injure the animal's skin, cause any kind of disturbance in the animal whatsoever, or impose any limit on movements necessary for its normal activities. For this reason it has not been found practicable to fasten the sanitary device used on the dog to the capsule wall, since this could lead to the displacement of the obturator and so disturb the normal working of the device.

In the literature (Pavlov, 1952; Haberland, 1934) methods have been described which envisaged a rigid fastening of animals during short term experiments (particularly acute ones). Clearly, these methods could not be used in our experiments. It was necessary, therefore, to design and test a number of other methods which adequately fastened the dog in the capsule and yet provided the freedom of movement necessary when a number of sensors for registering the animal's functions were attached to it.

A special fabric harness was designed which consisted of two basic parts which are put on the pectoral and pelvic girdles of a dog and referred to here as the jacket and shorts, respectively. The harnesses described are applicable both to bitches and dogs. The jacket and shorts have three openings each. In the jacket there are openings for the head and front paws, in the shorts there are two openings which are for the hind legs and a third (large one) for the tail and the duct of the sanitary device (Figures 1, 2 and 3). Pipings made of soft spongy rubber were sewn into the rim to protect the body against any injury. The jacket and shorts are provided with straps which surround the trunk with two rings: on the pelvic and pectoral girdles. The other two straps, on the right and on the left along the back, connect the jacket with the shorts. Where the annular and longitudinal straps cross, metallic rings are sewn in, to which are attached the snap links of the fastening chains. Along

the median line of the stomach, a rubber strap is passed for connecting the jacket with the shorts. This is sewn to the shorts beforehand and already put on the animal so that the strap length may be accurately adjusted. The free ends of the straps projecting from the buckles are sewn to the underlying harness.

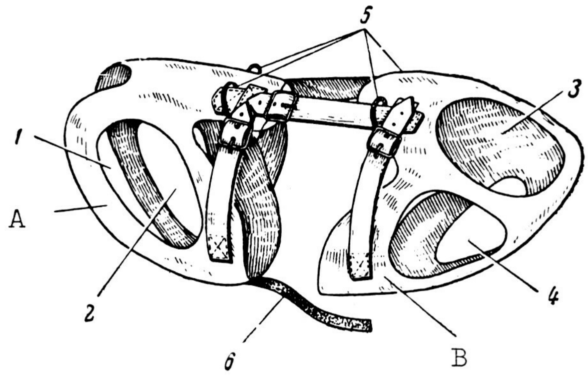


Figure 1. Diagram of the Fabric Harness

A - "Shorts"; B - "Jacket"; 1 - Opening for Tail and Duct; 2 - Openings for Hind Legs; 3 - Opening for Head; 4 - Openings for Front Paws; 5 - Rings; 6 - Rubber Tape

Complete sets of harness for dogs, as well as the sanitary device, were made in three standard sizes. The clothes are cut and sewn so that maximum conformity with the shape and dimensions of the dog may be achieved, and so that as few folds and seams as possible are produced, especially of those parts of the harness facing the body of the animal and capable of injuring its skin. This is particularly important in prolonged experiments. Fabric harness, in addition to its basic function of

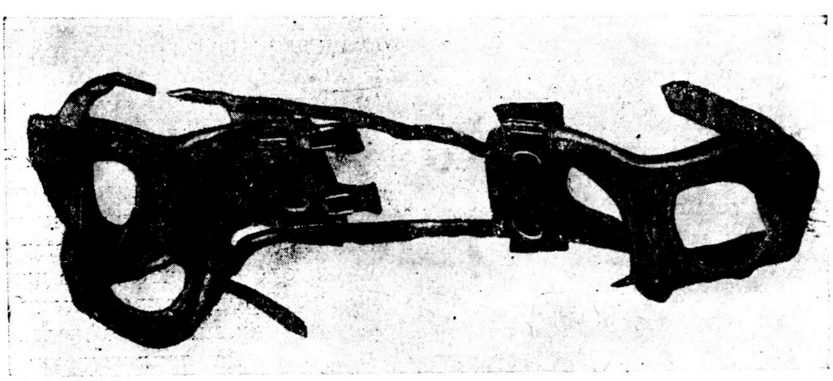

Figure 2. Fabric Harness

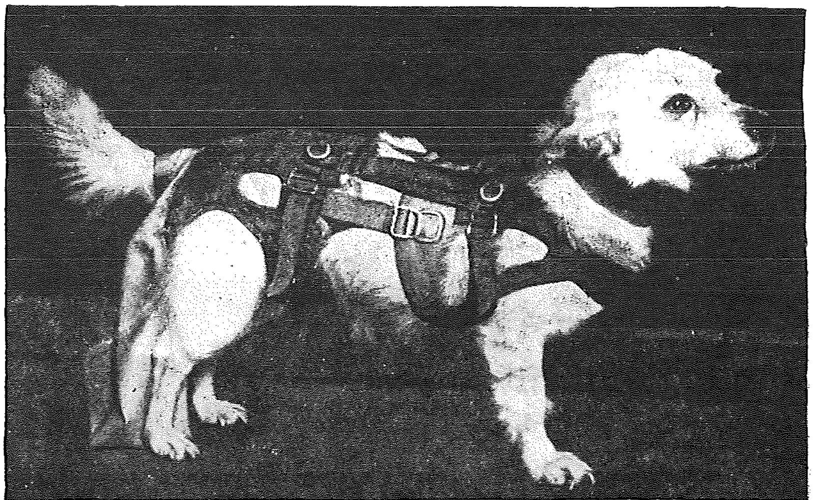

Figure 3. Fabric Harness Placed On the Dog Al'bina over the Sanitary Device (ASU-1)

fastening the animal in the capsule, contributes also to a better and closer adherence of the sanitary device to its body.

Small chains for immobilizing the dog in the capsule are attached at one end by means of snap links to the rings on the fabric harness. The other ends of these small chains run respectively forward and backwards to vertical metallic plates, behind the dog in the corners of the compartment and in front of the dog at the level of its head or neck. The plates have a number of openings to which the chains may be attached in accordance with the dog's height, and in such a way that the dog may freely lie down, get up and accept food and water from the automatic feeders (Figure 4). The front chains are somewhat longer than the rear ones, giving the animal a wider range of movement. The rear chains prevent the dog from moving forward and dragging the duct from the urine and faeces receptacle which is connected with the sanitary reservoir. However, the chains permit the animal to take up the position necessary for defecation.

Sensors for registering changes in physiological functions are placed on the animals. These include: a respiration sensor; cable from the motor activity sensor; sensor registering changes in arterial pressure and pulse rate; as well as grafted electrodes registering the animal's electrocardiogram. The respiration sensor is fixed on the chest and abdomen of the animal by means of rubber straps with buckles. In order to prevent a backward displacement of the respiration sensor, it is sewn in a few places, where there is no active layer changing its electrical resistance, to the overlying rear edge of the sanitary "brassiere". A certain average tightness of the rubber straps is chosen at which the respiratory movements of the animal give maximal resistance fluctuations.

The potentiometric motor sensor is fixed on the rear wall of the regenerating unit. The cable of the sensor is tied to the upper buckle of the sanitary "brassiere" so that its length permits registration of all the dog's movements to one side or the other, and so that the animal's forward or backward movements are not limited by the length to which the cable can extend but only by the fastening chains. Otherwise, the animal may break the cable or in its maximum backward movement its motion may not be registered.

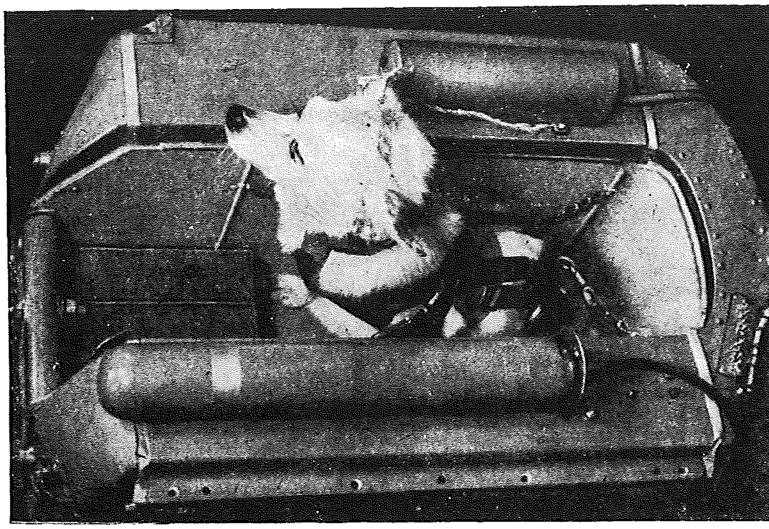

Figure 4. Fastening of the Dog Al'bina into the Compartment by Means of the Fabric Harness and Chains

The sensor for the determination of the arterial pressure is in the form of a rubber cuff which is contained in a plastic case consisting of two half cylinders with a hole for the skin fold. The sensor is put onto a neck skin fold which includes the carotid artery. The pressure in the cuff is varied cyclically by means of a special automatic inflator device which is connected to the cuff by means of a rubber tube. A miniature piezoelectric transducer acting as an oscillation sensor is mounted inside the cuff or anywhere along the lumen of the rubber pipe connecting the automatic device to the cuff. Automatic registration of the arterial pressure of the animal placed for many days in a pressurized capsule is carried out with considerable difficulty. Special conditions must be fulfilled for the proper attachment of the sensors to the animal and to the auxiliary parts of the apparatus: 1) the cuff of the sensor, together with the plastic case, must not be displaced and so pull at the flap during the dog's movements, otherwise injury or damage to the flap might occur; 2) it is necessary that the rubber tube passing to the sensor should not restrict the freedom of movement of the animal's head; 3) the rubber tube and the sensor should be protected from rubbing against the edges of the surrounding metallic objects, and the paws and teeth of the dog.

A special collar was designed and made for the protection and fastening of the sensor, and the rubber tube passing from the sensor to the automatic pressure device (Figures 5, 6 and 7). The collar was made from a wide (60 mm) strip of rubberized fabric. The inner (facing the animal) surface was covered by a layer of soft fabric, while the outer one had a closely woven cloth glued to it. Both ends of the collar had holes and loops of cord for lacing together, and in addition one had a valve. This permits the perimeter of the collar to be adjusted to the individual size of the dog. In critical experiments it was found desirable not to lace the ends together but to sew them to the lateral surface of the animal's neck.

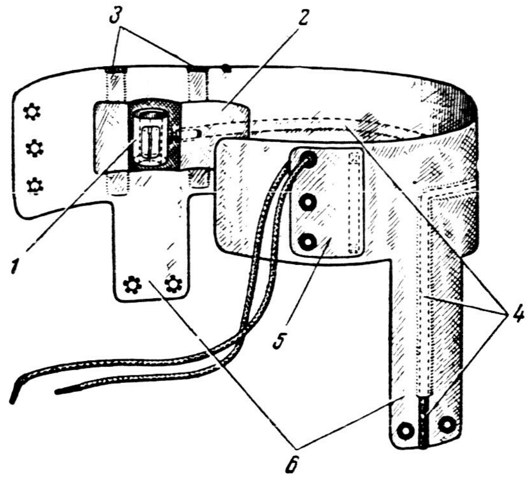


Figure 5. Sketch of the Collar

1 - Sensor; 2 - Tongue; 3 - Piping of Sponge Rubber;
4 - Rubber Tube; 5 - Valve; 6 - Side Pieces

Along the median line of the dog's body from above and below the collar, two side pieces are running (the upper is longer than the lower) which are freely attached to the "brassiere" of the sanitary device so that they do not interfere with the movement of the animal's head.

In the section of the collar facing the neck skinfold, the two layers of the collar (the soft layer of the fabric and the rubberized fabric) nearest to the body of the dog are cut away to a rectangular shape which corresponds in width to the sensor cylinder, although in length it is somewhat longer than the cylinder. The outer layer of closely woven fabric which is rather loose in this place thus forms a pocket containing the sensor. From the edge of the pocket runs a long tongue made of quite soft, rubberized fabric, which is passed under the dog's neck skinfold

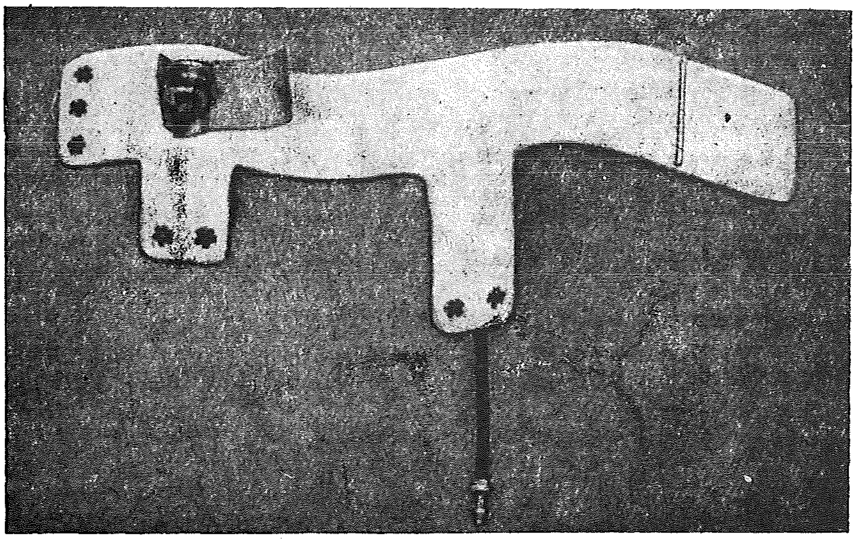

Figure 6. Collar (Viewed from the Inside)

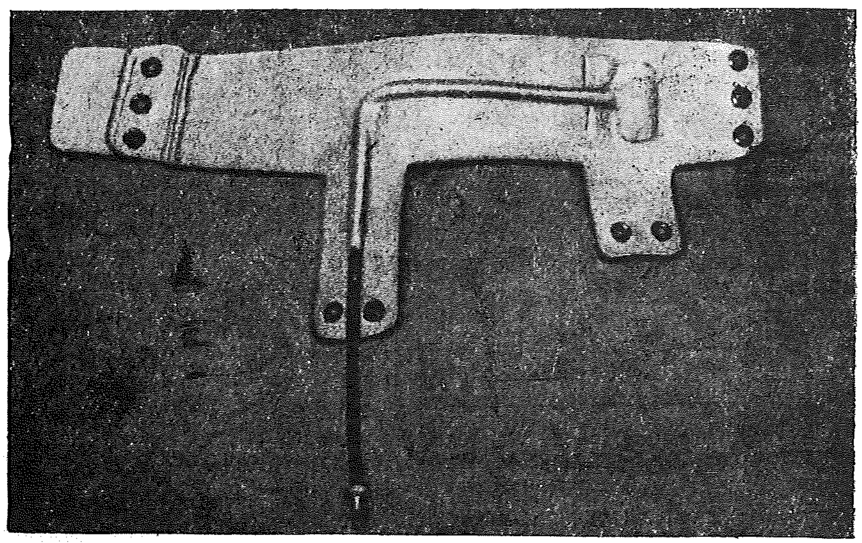

Figure 7. Collar (Viewed from the Outside)

and underneath the sensor, so as to protect the skin of the animal from any irritation caused by the protruding parts of the sensor (e.g., by the loops of the half cylinders) during prolonged experiments. The end of the tongue is sewn to the collar in order to avoid displacement.

On both sides of the rectangular pocket and along the whole width of the collar under the layer of soft fabric are placed pipings of soft, spongy rubber. These pipings help to fasten the sensor and prevent its lateral displacement. In addition, the piping lifts the edges of the collar above and below the sensor and so protects the sharp bend of the neck skinfold which lies between the sensor and the surface of the neck.

A rubber tube is attached to the connecting teat of the sensor's cuff which is fixed to the neck skinfold. The rubber tube runs from the sensor pocket between the external fabric and the central rubberized layer of the collar, and passes round the collar to the level of the mid-vertebral line of the animal; here it turns through $90^{\circ}$ and continues on the upper side piece of the collar (Figures 5, 6 and 7). To give the rubber tube the required shape when the collar is being made, the former is vulcanized in the corresponding bent position. The surplus end of the tube protruding from the collar is cut off when the animal is prepared for the experiment. The end of the rubber tube is then joined through a connector with the vacuum rubber tube from the automatic inflator device which inflates the cuff.

In one modification, the miniaturized piezoelectric sensor for registering the pulsation of the vascular wall was placed inside the rubber cuff which was put on the neck skinfold. In this case the electric leads from the sensor ran with the rubber tubing in between the layers of the collar (this modification was used in the experiments with Layka). In another modification, a piezoelectric or thermoanemometric sensor for registering changes in pulse rate was connected to the end of the rubber tube. This rubber tube came out of the collar at the back of the animal. A vacuum tube passing from the automatic inflator device was put on the other side of the sensor. The removable pulse rate sensor was placed on a hook of the "brassiere" of the sanitary device in a specially protected pocket (Figure 8). Electrical leads from the removable sensor run together with the vacuum tube. The length of the tubes and the points where they are connected should not limit the movements of the dog's head.

Leads from the electrodes are fastened by cords to the body of the animal and run through an opening pierced in the sanitary device to its surface, they are sewn with thread to the "brassiere" and joined to a bundle of leads from the respiration sensor and the vacuum tube. A small knot is made in the right hand lead. The bundle leads, together with the vacuum tube, pass between the fabric harness and the sanitary device, and then run through the opening for the tail in the duct of the urine and faeces receptacle. The leads are soldered to the corresponding tags placed at the rear of the regenerating units. The part of the leads between the animal and the tags, as well as the vacuum tube, should be sufficiently long to prevent either the displacement of the sensors or the leads from breaking during the movements of the animal.

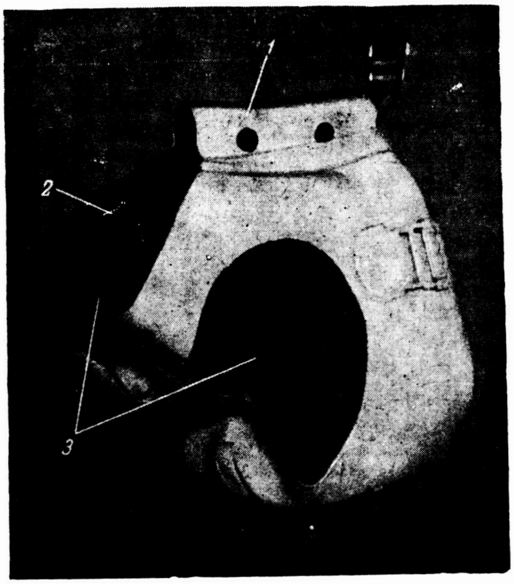


Figure 8. "Brassiere" of the Sanitary Device with a Special Protective Pocket at the Back to House the Removable Pulse Rate Sensor

1 - Protective Pocket; 2 - Hole for the Head; 3 - Holes for the Front Legs

Repeated and prolonged (up to 20 days) testing of the above described methods in pressurized cabins gave good results and showed that the techniques are fully adequate to meet the experimental requirements. The method just described, of fastening an animal in a pressurized cabin and attaching the sensors to its body, was used in the experiment with Layka in the second artificial satellite and served as the basis for developing the methods used subsequently in biological experiments with dogs on spaceship-satellites.

Summary

11694

A fabric harness has been developed for affixing sensors to the body of a dog during space flight. It consists of two pieces which fit over the head and forelimbs, and hindquarters, respectively, and which are joined together by straps. A pocket in the neckpiece accommodates the sensor for recording pulse rate and arterial pressure. The harness is fixed to the capsule wall by means of chains which permit free movement of the animal, and at the same time prevent traction on the sensor leads.

The harness was tolerated by animals for periods of up to 20 days, and was used on the dog Layka during the second space flight.

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# PROVIDING DOGS WITH FOOD AND WATER UNDER SPACE-FLIGHT CONDITIONS

I. S. Balakhovskiy, L. I. Karpova and S. F. Simpura

The problem of feeding experimental animals during biological studies on rockets and balloons which last for only a few hours, or certainly not more than 10 hours, does not arise.

The preparation of an animal for a prolonged flight on an artificial Earth satellite which lasts a number of days, however, necessitates solution of this problem. During such flights the animal should be provided with food in the quantity required to replace the energy losses of the organism, retain the protein equilibrium and saturate the organism with vitamins throughout the whole duration of the flight. However, if the flight does not exceed one or two weeks, the most important is replacement of the energy losses, since a negative nitrogen equilibrium, and even more lack of vitamins, will not have any substantial effect. The feeding system should be as compact as possible so that the reserve of foodstuffs and water and also the volume of the excreta should be minimum. From this point of view it is convenient to enrich the food as much as possible with fats and carbohydrates and lower the quantity of proteins, since during oxidation of foods which do not contain proteins carbon dioxide and water are formed. Carbon dioxide is evolved in the form of gaseous products and the water formed compensates the corresponding decrease of water reserves taken from Earth. Nitrogenous products are less convenient from this point of view since in order to reject the recrements, in particular urea, additional quantities of water are required. It is obvious that the food should be of such a shape that its presentation to the animal can be fully automated.

The quantity of water issued should satisfy the requirements of the animal at the various temperatures which may occur in the capsule. However, any water not consumed must not be spilled since it could damage various instruments.

The purpose of the present work includes determination of the necessary quantity and periodicity of food and water supplies, the form in which it should be supplied and also the drawing up of concrete plans for the space flight. The equipment necessary for the above purposes was constructed by a group of engineers of one of the factories manufacturing biophysical instruments.

## Determination of the Energy Losses

The calorific value of the food should correspond to, or exceed, the possible energy losses of the animal. For that reason determination of the calorific value is one of the more important problems in the development of a rational diet. Although the energy losses in dogs have been studied frequently, in most cases only the fundamental metabolism was studied and not the gaseous exchange, the value of which from the practical point of view is much greater, since the animals, during the flight, are in conditions entirely different from those in which the metabolism is usually measured.

Literature data could be used as starting points but they required additional checking on a group of experimental animals. One of these animals, who was ultimately selected for the satellite flight, was experimented upon in conditions which were as closely similar as possible to those encountered during the flight.

The Douglas Holden method was used in which the expired air was taken via a mask and collected in a rubber bag. At the beginning the energy losses of eight experimental animals were determined. These animals were placed in troughs in an insulated chamber. The temperature of the ambient medium was 18-20$^{\circ}$ C. Each investigation took 4 hours, during the night the dogs were kept in a vivarium and during the day of the experiment ordinary food was served.

In spite of rigorous observation of identical experimental conditions, considerable variations in the quantity of gas exchange were observed, which could be explained as individual differences between the animals and by different degrees of their activities. The results are shown in Table 1.

The values of the gas exchange were checked in addition in experiments on dogs who were immobilized and placed for a number of days in various models of pressurized capsules. Each capsule as a rule contained two dogs. These animals were dressed in their immobilizing and sanitary apparel and received food and water from the automatic food dispenser. Although the experiments were carried out in an ordinary laboratory and no special measures were taken to isolate the animals from the external environment and to imitate other flight factors, it may be said that these conditions were very close to those of the space flight. The results of the experiments are given in Table 2.

The gas exchange values obtained in these experiments coincide sufficiently well with the literature data (Slonim, 1952). As may be seen from Tables 1 and 2, the average quantities of oxygen required are substantially the same for animals placed in special troughs or immobilized

Table 1. Gas Exchange in the Experimental Dogs Determined under Laboratory Conditions

| Name | Wt, kg | No. of experi-ments | Oxygen consumption, l/hour | Oxygen consumption, per 1 kg body weight, l/hour | Energy losses during 24 hrs, kg/body weight |
|---|---|---|---|---|---|
| Pestraya | 7.5 | 45 | 4.7 | 0.626 | 73.6 |
| Umnitsa | 6.4 | 86 | 5.8 | 0.906 | 107.9 |
| Sedaya | 5.0 | 32 | 3.6 | 0.720 | 78.8 |
| Sil'va | 5.3 | 30 | 3.2 | 0.604 | 66 |
| Belka | 3.8 | 21 | 3.0 | 0.800 | 90.4 |
| Nora | 4.8 | 17 | 4.1 | 0.854 | 92.8 |
| Chernushka | 5.1 | 23 | 3.9 | 0.764 | 84.7 |
| Layka | 5.1 | 23 | 3.4 | 0.670 | 79.8 |

in the models of the capsules. However, in one group of animals it was possible to observe a regular decrease in the gas exchange according to the degree of training in the capsule. These animals were calm and tolerated well the immobilization. The other group, on the other hand, did not tolerate the immobilization well and their oxygen requirements kept increasing.

The values of the gas exchange, which varied within 400-700 kcal/day, should be looked upon rather as an upper limit of the energy

Table 2. Gas Exchange in the Experimental Dogs, Placed for a Long Time in an Immobilized State in Models of Satellite Capsules

| Name | Wt, kg | No. of experi-ments | Oxygen consumption, l/hour | Oxygen consumption, per 1 kg body weight, l/hour | Energy losses during 24 hrs, kg/body weight |
|---|---|---|---|---|---|
| Al'bina* | 5.0 | | 3.6 | 0.720 | 100.0 |
| Sil'va | 5.2 | 23 | 4.0 | 0.770 | 90.5 |
| Belka | 3.9 | 21 | 3.4 | 0.870 | 93.2 |
| Nora | 4.9 | 15 | 3.4 | 0.632 | 72.0 |
| Chernushka | 5.9 | 17 | 3.3 | 0.560 | 75.9 |
| Layka | 4.9 | 22 | 3.3 | 0.673 | 72.3 |

*The experiments were carried out in a model of a satellite cabin designated for one dog.

requirements of the animals and not as a factual or de facto requirement during the time of flight, since during determination of the gas exchange according to the Douglas Holden method the expired air is collected by means of a mask and the putting on of the mask itself excites the animal and increases the gas exchange. In all probability, not only in the conditions of space flight when the quantity of external irritants is minimal but also in the ordinary laboratory experiments lasting for a number of days, the consumption of oxygen should be lower. For that reason the data obtained should be checked in the experiments with prolonged feeding of animals with food mixtures of known calorific content.

## Determination of Food Requirements

In a special series of experiments, the minimum quantity of food required to sustain the animals in a satisfactory condition during a 20-day long test in conditions similar to our experiment was determined. For that purpose representatives of various weight groups were selected among the animals - Umnitsa weighing 7300 g, Al'bina weighing 5700 g, Mukha weighing 4300 g, and they were kept for 20 days in a vivarium in an immobilized state and received per day: Umnitsa - 100 g, Al'bina - 80 g and Mukha - 50 g of briquettes consisting of meat, biscuits and fat of a total calorific content of 500 kcal per 100 g. The animals were weighed every day, and the quantity of water drunk was also weighed. Their general state was also examined daily. The experimental results are given in Figure 1. As may be seen from this figure, there was no progressive loss in weight during the 20 days of the experiment and the general state of the animals remained satisfactory.

On the basis of the above, certain preliminary indications about the required lower limit of the calorific value of the food were obtained. This was deduced to be 500 kcal per day for dogs weighing more than 7 kg; 400 kcal per day for dogs weighing 5-7 kg and 250 kcal for dogs weighing less than 5 kg.

Furthermore, during development of the dietary mixtures and the concrete forms in which they should be issued as well as during the training of the animals, all these experiments were frequently repeated. As a rule their duration was 7 days with a calorific value of the food of 450-500 kcal per day. Altogether approximately 40 tests were carried out. If the animals ate all the mixture their weight did not decrease.

## Determination of the Water Requirements

Determination of the water requirements of the animals is considerably complicated since it depends on the state of the thermal regulation, which in turn depends on a number of medium parameters, namely,

temperature, humidity, air speed, temperature of the capsule walls and type of immobilization of the animal in the capsule.

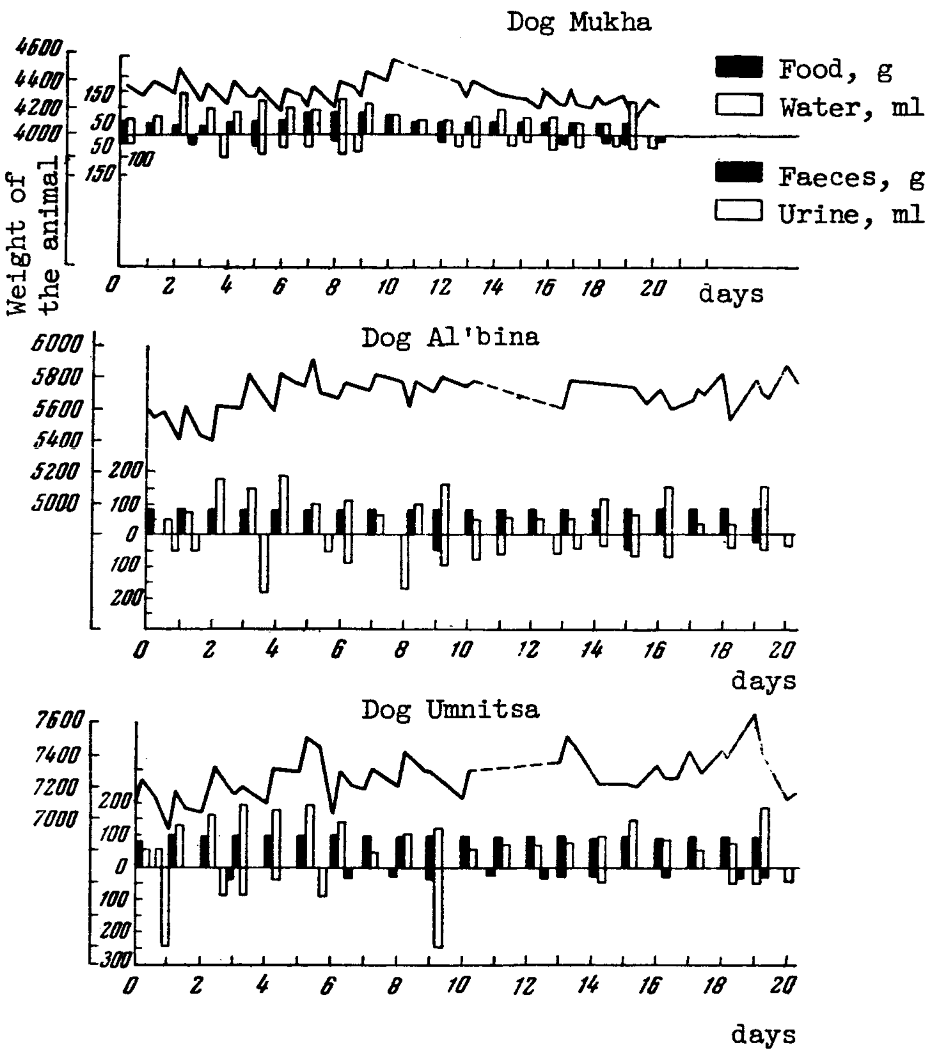


Figure 1. Changes in Weight, Quantity of Accepted Food and Excreta of Dogs Fed with the Experimental Ration

Dogs have no sweat glands. For that reason during overheating they begin to breathe frequently and shallowly, removing the excess of heat by evaporation of saliva from the mucous membranes of the mouth and the upper respiratory passages. Initiation of this mechanism is immediately noticeable when observing the animal. The above described experiments were carried out in conditions under which this particular mechanism did not appear.

The animals were placed for a number of days in troughs to which they were immobilized by means of fabric apparel. The surface of the troughs was covered with felt. The animals were fed with briquettes containing powdered biscuits, powdered meat and fat. The quantity of water consumed was determined each day (the animals received a bowl of water twice per 24 hours, the water being at room temperature), as well as the quantity of urine and the weight loss. The results of the gas exchange have shown that the respiratory coefficient of the experimental animals was approximately 0.7. Since carbon dioxide has a 1.37 times higher specific weight than oxygen, the changes in the weight of the animals could not be due to the fact that the losses of carbon dioxide exceeded the amount of oxygen absorbed. Hence, by weighing the animals within exactly fixed intervals of time it was possible to determine the water loss via the lungs.

The quantity of water drunk freely by three animals of various weight groups is given in Figure 1. As may be seen, there were considerable variations in the quantity of water drunk. The average requirements per day was 120 ml. The experiments carried out on other animals gave similar results.

The quantity of water lost through the lung differed also quite considerably (from 0.4 to 1.4 g) and comprised on average 0.8 g per 1 kg of body weight per hour. For animals weighing 6 kg, this corresponded to a loss of 115 g of water vapor per 24 hours. As a rule the urine was of a saturated yellow and orange color with a specific weight more than 1.025. The quantity of urine was up to 250 ml. Insofar as the evolution of water exceeded the amount taken in, the additional quantity was clearly formed as a result of burning the hydrogenous foodstuffs.

On the basis of these experiments it is possible to conclude that under conditions of unstrained thermal regulation the water requirement of a dog comprises less than 200 ml per 24 hours. With increasing temperature this requirement may considerably increase.

## Construction of the Automatic Food Dispenser

Among the problems aiming at providing the necessary environmental conditions for the animals during space flight one of the most complex is the design of a method of feeding the animal with water. The customary troughs are not applicable in these conditions since during weightlessness water, as well as any other liquid, will spread itself all over the capsule and the animals will be unable to drink it. For that reason various wicks, teats, feeding troughs with a perforated bottom, etc., were suggested. The efficiency of these devices, however, was highly doubtful and to check their working under the conditions on Earth is practically impossible. In addition, all of them required some sort of a dosing device for securing a daily ration.

Taking into consideration the imperfections of the systems which supply water to an animal in the liquid state, we decided to make a combined automat which would supply water and food in the form of a unified gelatinous mass. Such a system permits dispensing with the relatively complex dosing apparatus and reduces to a minimum the possibility of spilling the food in the capsule, and at the same time solved the problem of eliminating the unconsumed food and water.

The combined automat had only one deficiency as compared with the system of separate food and water supplies, namely, it was more bulky. Although the dimensions and weight are of considerable importance during space flights, the other advantages of the combined method were so great that they fully compensated this deficiency.

The automatic food dispenser, designed and fabricated in a factory, consisted of a frame to which were attached, by means of two strips, rectangular containers with food connected together with hinges. The volume of each container was 330 ml. This chain of containers was moved by means of a special electric motor. When the container approached the front end of the automat its lid engaged in the protrusions on the frame and opened, after which the movement ceased. The next electrical signal again actuated the container, the lid of the jar was automatically shut and the movement continued until the next jar appeared in its place and opened. After this the movement ceased again until the next signal was received.

If signals actuating the automat were sent once per 24 hours, the dog had enough time to eat its food completely. However, if for some reason the mixture is not completely consumed, the remnants are left in the jar with a tightly shut lid and are moved to the lower part of the automat; in this way they are substantially isolated from the rest of the capsule. Prior to the experiment the feeding mixture was poured into the jars, the lids were hermetically shut and the whole system was sterilized in an autoclave for 40 minutes at 115° C. In order to determine the permissible time of storage, the feeding mixture was kept in the jars after the sterilization at a temperature of 18-20° C for a period of two months. The subsequent bacteriological tests showed that the mixture retained its sterility.

## Composition of the Feeding Mixture

During the preliminary tests, a number of feeding mixtures were approved. The most suitable was a mixture with the composition given in Table 3.

Table 3. Composition of the Daily Quantity of the Feeding Mixture

| Product | Quantity, g | Proteins, g | Fats, g | Carbohydrates, g | Calorific value, kcal |
|---|---|---|---|---|---|
| Meat, beef (lower than average feeding) | 80 | 15.89 | 2.74 | - | 90.6 |
| Kitchen fat of mixed origin | 30 | - | 28.36 | - | 263.7 |
| Oats, groats "Hercules" | 10 | 0.91 | 0.6 | 6.1 | 34.3 |
| Pork sausage semi-smoked | 20 | 3.84 | 4.5 | - | 57.6 |
| Agar-agar | 2 | 0.06 | - | 1.8 | 7.6 |
| Water | 188 | - | - | - | - |
| Polyvitamin preparation containing $A_1$, C, $B_1$, $B_2$, PP | | | | | |
| Total | 330 | 20.5 | 36.2 | 7.9 | 453.8 |

As can be seen from this table, the dog receives each day 20 g of proteins, 36 g of fats, 8 g of carbohydrates and 250 g of water. This diet has a total calorific value of 450 kcal. The main contribution to the calorific value is from the fats, while proteins contribute only 18 percent of the total calories. This corresponds to the recommendations of N. M. In'kov (1958) who considers that proteins should cover not less than 12 percent of the energy losses of the animal. The relative poverty of the ration in nitrogenous materials makes it possible to reduce the quantity of nitrogenous recrements which are expelled together with the urine.

The semi-smoked pork sausage was added in order to improve the taste of the food, 0.6 percent agar-agar was added to produce the gelatinous consistency in the food. During short experiments (lasting for only a few days) there is no need to provide the animal with any vitamins, since during such a short period of time there will be no deficiency. However, since before the flight the dogs underwent special training, they were all kept for a long time on an experimental diet and for that reason the addition of vitamins was justified.

A special test was carried out to check the mixture, during which five dogs were kept in cages in the vivarium and fed daily with the mixture according to the formula in Table 3. The weight of the dogs and the quantity of the food eaten were taken daily and the general state of their

health was also inspected. Not one of the dogs lost weight and two of them actually gained some 400 g. Clinically all the animals were in good shape and were quite calm. The positive result of this test, and the agreement between the calorific value of the feeding mixtures with the amount of energy losses determined from the data of the gas exchange, made it possible to start the preparation of a practical system of feeding the animals during space flight in a satellite.

## Training the Animals for Feeding During the Time of the Flight

During the time of a satellite flight, as well as during the training and control experiments, the animals were placed under extraordinary conditions, for their movements were limited by the small volume of the capsule and their immobilizing apparel, their defecation and urination was made difficult, their food was not served in an ordinary bowl but had to be reached from a jar of the automatic food dispenser placed below the level of the "floor". The activity of the animal during feeding may be lowered also as a result of the noise of the electric motors of the apparatus, the clicking of the relays, etc. For that reason, although all the animals in the conditions of the vivarium transferred to feeding with the mixture without any difficulty, they had to be specially trained in order to feed normally in the experiments imitating the flight in a satellite.

As a rule the training was carried out in three stages. During the first or "general" stage, which was described in detail by O. G. Gazenko and V. S. Georgiyevskiy (1961), the animals were trained to wear the immobilizing apparel, sanitary devices, etc., after which their specific training in feeding was undertaken. This was carried out in two stages. During the first one, the animals were immobilized for four days and nights in a special stand and were fed from the automatic dispenser (Figure 2). During this time the animals were without the sanitary devices or sensors of the physiological parameters; external irritants were also eliminated as far as possible. During the second stage (6 days), the animals were immobilized in the same stand but this time wearing the sanitary devices.

When these two training stages had been completed the animals could participate in experiments in which, as far as possible, all the conditions encountered during flight in a satellite were imitated. The efficiency of training the animals and its necessity is illustrated in Table 4 in which the experimental results on dogs are given. These results are based on dogs drawn into the experiments for the first time. When fully trained, the animals endured well the tests and did not lose any weight, or lost it only insignificantly. However, if the training was incomplete or if the time between the various stages was not very long, the dogs were found to lose weight.

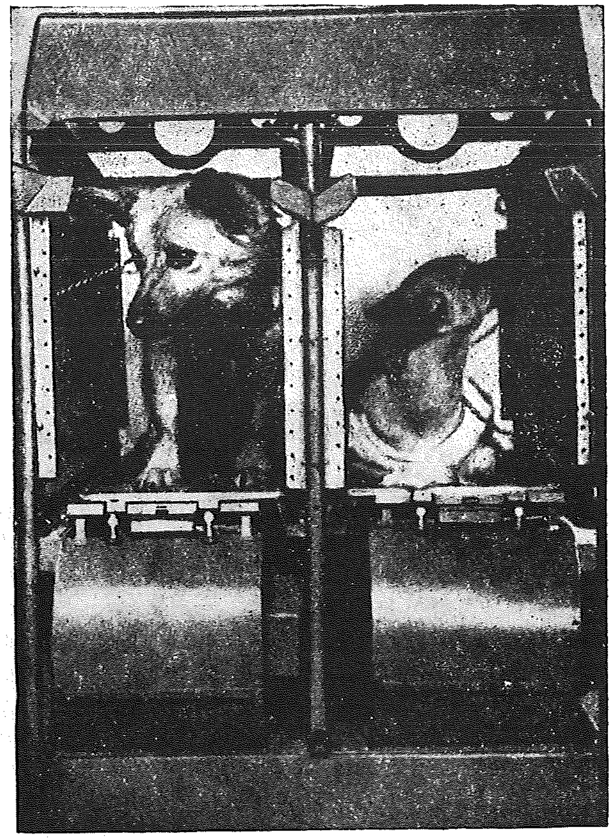

Figure 2. Dogs during Training. The Automatic Dispenser May Be Seen at the Bottom

During repeated tests various dogs behave differently. In some of them the training was retained for a very long time, others required repetition of the full cycle and of training in feeding. It should be said that the above method must not be understood as completely absolute and applicable to the same degree to all dogs. For some of them the learning is much easier and for others more difficult. Thus, it may be seen from Table 4 that in the case of Il'va and Laska the times between the termination of the training and the experiment were almost the same. During this time the first lost all her habits while the second did not.

Since the animals taken for the experiment without preliminary training lost weight and were feeding poorly, the training of all the animals according to the full program is considered necessary, although it is true that for certain animals this could be avoided and a much shorter training program can be adopted, if it were possible to determine which dogs become accustomed more easily to the conditions of the experiment. Insofar as the assessment of such criteria is in itself a complex

Table 4. Change in Weight of the Dogs during Experiments Simulating Flight in a Satellite

Dogs not fully trained or dogs tested a considerable time after completion of the training

| Name | Wt, g | Training wearing the sanitary device | Feeding Training | | Experiment imitating flight in a satellite | |
|---|---|---|---|---|---|---|
| | | | Duration | Change in wt | Duration | Change in wt |
| Gil'da | 6720 | None | 26 Jan-3 Feb | -320 | 4-8 Feb | -400 |
| Il'va | 4400 | 7-22 Dec | 24-31 Dec | +100 | 4-8 Feb | -750 |
| Lisichka | 4200 | 4-8 Apr (only the 1st stage) | 23-26 Mar | +100 | 23 Jun-1 Jul | -190 |
| Irma | 4880 | None | 7-14 Jan | -320 | - | - |
| Tiksa | 4880 | None | 14-21 Jan | -250 | - | - |
| Dogs that underwent full training | | | | | | |
| Sil'va | 4900 | 20-30 Mar | 22-30 May | +40 | 12-17 Jun | +100 |
| Laska | 5700 | 13-19 Mar | 6-12 May | -20 | 12-17 Jun | -50 |
| Chayka | 5150 | 21 Feb-2 Mar | 21-30 Mar | +100 | 5-18 Apr | +100 |

scientific problem, we limited ourselves to following the scheme of training which is sufficient for all the dogs.

Some dogs, although they ate all the food given to them, would not do this in one go as most of the animals did but over a long period, eating most of it during the night. This is clearly connected with the fact that the animals were of the refractory type and the external irritants delayed their feeding reflexes. In one of the experiments a modified automatic dispenser was used in which the access to the food was opened to the animal only during 12 hours. It was found that the dog using this dispenser lost 180 g within five days, although before that she had gone through the whole cycle of training and withstood it well.

During the development of the feeding system and the training of the animals, 25 experiments in all were carried out imitating the flight in a satellite of 6-7 days duration. When the animals ate all their food they did not lose any weight. The data obtained make it possible to conclude that a daily ration of 330 g of the above described feeding mixture is sufficient for sustaining the dogs in a satisfactory state during the satellite flight.

Provision of the Dog Layka with Food and Water
during the Flight in the Second Satellite

Since the flight of the dog Layka on the second artificial Earth satellite was due to last for only seven days, it was possible to dispense with the use of any automatic food dispensing equipment and it was sufficient to open up, prior to the launching, the access to the entire quantity of food to insure maintenance of its life for this period. Such a system is considerably easier in terms of construction and gives economy in weight and dispenses with the electrical systems which actuate the apparatus during the time of flight.

In order to store and issue the food, a special trough, in the form of a tin-box of 2.7 liter capacity with a lid which was opened by means of an electric current, was used. Into the trough were introduced 2.5 liters of jelly prepared according to the following formula: 80 g of powdered beef, 80 g of powdered biscuits, 40 g of beef fats, 20 g of agar-agar, 1 g of salicylic acid, and up to 1 liter of water. The calorific value of this mixture was 1000 kcal.

The feeding mixture contained in the trough was simultaneously the source of food and water. After setting, the agar-agar jelly attained a mechanical strength which retained its wholeness during the pitching and tossing of the trough. The calorific value of all the food was approximately 2500 kcal. This corresponds approximately to the possible energy losses of the animal during a week. The quantity of water was in excess of 1.4 liters. This quantity was based on the consideration that its requirements did not exceed 200 ml per day, as was shown in our experiments.

This particular variant of the feeding system was tested on two animals, Umnitsa and Zhuchka. The bitch Umnitsa was placed in a nonimmobilized state in a cage. She received the gelatinous food in a quantity of 2530 g during eight days. Towards the end of this period the animal was in a satisfactory state. The bitch Zhuchka, who was placed during the experiment in a pressurized capsule for 8 days, was also fed with a gelatinous mixture which she received in the form of a single portion of 2.8 kg. At the end of the experiment the clinical state of the animal was fully satisfactory.

It was concluded from the above calculations and also from observations that, by using the above described methods, it is possible to secure satisfactory feeding of an animal during the flight on a satellite.

Prior to the rocket launching, the lid of the trough was opened electrically and the animal gained access to the mass of the feeding mixture.

## Provision with Food of the Dogs Belka and Strelka during Flight on the Second Spaceship

Belka and Strelka were accustomed to feeding with the mixture from the above described automatic dispenser according to the general program. At first the animals passed the training with the immobilizing apparel and the sanitary devices and then, during the next seven days, they became accustomed to feeding from the automatic dispenser. The dogs endured this preparation very well, almost without any changes in weight, Belka added 30 g, and Strelka lost 50 g.

Since, as has been mentioned before, dogs lose their habit of feeding from the automat, this particular training was repeated directly on the launching pad.

The feeding mixture was prepared earlier, placed in the containers of the automatic dispenser, covered hermetically with rubber washers and sterilized for 40 min at 115° C.

The first container mounted in each automatic dispenser was designated only for checking the working of the whole device and was not filled with the feeding mixture. The second container contained ordinary water which the animals should have drunk while still on Earth before the launching of the rocket. The remaining containers were filled with the feeding mixture. Since the duration of the flight was planned in terms of days and there were many food containers in the dispenser, the food was issued not once but twice during each 24-hour interval. Signals were received by telemetry on both occasions informing about the opening of the containers. It was also possible to see on the screen of the television that the animals were feeding.

When the spaceship landed it was found that of the containers opened first during the flight, almost all the food had been eaten - Belka left 20 g and Strelka left 105 g. The second containers were empty.

## Conclusions

1. The dry food mass containing 20 -30 percent fat and 70-80 percent proteins and carbohydrates in a quantity of 100 g per 24 hours provided all the requirements of dogs weighing up to 7 kg for a period of 20 days.

2. The water requirement for dogs of the same weight at a temperature up to 20° C was on an average 100-120 g and never exceeded 200 g.

3. The loss of water through the lungs at room temperature in the experimental dogs was found to be on average 0.8 g per kg body weight per hour with variations of 0.4-1.4 g.

4. Studies of gas exchange in dogs, carried out under the conditions of the described experiments, yielded energy loss values amounting to 400-650 kcal per 24 hours which are close to the data obtained by other authors.

Summary

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The authors have determined the amount of food and water required by dogs, weighing 5 to 8 kg, during space-flight conditions. In a preliminary study of energy requirements the oxygen consumption of 8 dogs ranged from 0.604 to 0.906 l/hr/kg, and the 24-hour energy expenditure from 66 to 107.9 kg/body weight. These figures did not change essentially when the animals were confined in a simulated space cabin. Three dogs kept under similar conditions for 20 days remained well and lost no weight on a daily diet of 50-100 g of a jelly containing meat, sugar and fat to a total calorific value of 500 kcal/100 g. The average daily intake of water was 120 ml, and the average rate of loss of water in the breath was 0.8 g/kg/hr. The construction of an automatic feeding apparatus is described, and also the regime used for feeding the dogs Layka, Belka and Strelka during their space flights.

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# RESULTS OF EXPERIMENTS CARRIED OUT IN SATELLITES

I. S. Balakhovskiy, O. G. Gazenko, A. A. Gyurdzhian,
A. M. Genin, A. R. Kotovskaya, A. D. Seryapin,
V. I. Yazdovskiy

Considerable material has accumulated as a result of Soviet and foreign studies on biological effects caused by various factors encountered in space flight. In these investigations, airships (balloons) and high altitude rockets were employed. Airships have the advantage that animals can be maintained in them for a long period of time, but unfortunately the altitudes to which they can reach are relatively low and such factors as acceleration or weightlessness cannot be studied in them. Experimental conditions on airships were found to be suitable for studying the effects of cosmic radiation on animals and also for general testing of high altitude equipment (Henry et al., 1952; Chase, 1954).

Rockets, which can reach considerably higher altitudes, enable the study of the effects of a wider range of basic flight factors to be made (Balakhovskiy, Malkin, 1956). In the case of rocket experiments, the experimenters were particularly interested in the state of animals during the two different stages of the flight trajectory, viz. during the so-called powered stage, i.e., the part during which the vehicle is subjected to acceleration, vibration and noises of the rocket engines and the free flight trajectory, during which dynamic weightlessness is established.

V. I. Yazdovskiy, A. V. Pokrivskiy and A. D. Seryapin (1957), B. G. Bugrov et al. (1958) studied the effects of rocket flight on unanesthetized dogs. The authors concluded that the animals withstood flight at altitudes of 100-110 km without any serious disturbances being produced in their basic physiological functions. At the same time, it was shown that during the powered section as well as during the reentry of the capsule into the denser atmospheric layers, a certain increase in the arterial blood pressure, pulse rate and respiration rate were observed. During the free flight, under conditions of weightlessness which lasted for approximately 3 minutes, there was a tendency for the arterial blood pressure to be reduced and lowering of respiration and pulse rates were also observed. Similar data were also obtained by Henry, Ballinger, Maher and Simons (Henry et al., 1952). In this work, experiments were carried out on small anesthetized monkeys in rocket flights reaching 60-120 km. These authors did not detect noticeable disturbances in the physiological functions of the experimental animals but they did observe a tendency for the maximal and minimal arterial blood pressures to be lowered during the periods of weightlessness. According to these

investigators, the physiological functions of the experimental monkeys differed very little during the flight from their original state. It is evident that brief rocket flights into the upper layers of the atmosphere are satisfactorily endured by these animals. As far as certain differences in the physiological reactions of the dogs and the monkeys are concerned, these could be explained by species differences and also by the fact that the monkeys were anesthetized while the dogs were not.

A. M. Galkin et al. (1958) carried out experiments in which two dogs, one anesthetized, were placed in a rocket at altitudes of 200-212 km. The conditions of rocket flight at an altitude of 212 km were much more severe with respect to the intensity and duration of the action of the various factors than in all the earlier experiments. The duration of weightlessness was now extended to six minutes. In spite of that the authors did not notice any acute disturbances in the behavior and physical functions of the dogs. During the active part of the flight, pulse and respiration rates as well as the arterial blood pressure of the nonanesthetized animal were as a rule considerably increased. At the beginning of the period of dynamic weightlessness, physiological indicators remained at a higher level than the original ones but showed a tendency to decrease. The return to the original level took place within 5 to 6 minutes from the beginning of the period of weightlessness. During the whole period of weightlessness the pulse and respiration rates and the arterial blood pressure of the anesthetized animal did not vary significantly from their original levels which is in good agreement with the observations of Henry, Ballinger et al. on anesthetized monkeys.

In this way, it was shown that when anesthetized animals were used there were no noticeable changes in these basic physiological parameters. Changes observed in nonanesthetized dogs generally present a picture of physiological disturbances, accompanied by a well-expressed orientational and defensive reaction. However, in the experiments carried out during flights to an altitude of 212 km, in addition to the above symptoms there was a sufficiently clear and specific effect of accelerations during the powered stage of the flight as well as during the reentry of the rocket into the dense layers of the atmosphere.

On the basis of the collected material, it was considered fully possible to send the animals in rockets to even higher altitudes. The accumulated information was sufficient to assess critically various methods and systems of securing for the animals optimal conditions during the flight. In some cases, in order to continue with the experiments, it was necessary to modify considerably the existing systems, and in others to design new ones.

The results of the above mentioned and other experiments made it possible to prepare a biological experiment to be made in an artificial satellite which would be placed in orbit round the earth. This type of

experiment was of great interest. In contrast to the high altitude rockets which are used for examining the upper layers of the atmosphere, artificial satellites permit the study of the behavior and the state of animals during prolonged flight at an altitude of 100-1000 km from the surface of the earth. Consequently, artificial satellites can be used to produce conditions which, from the biological point of view, are very near, if not identical to those encountered during cosmic flight.

In this way, it was possible to study the effects of prolonged acceleration, noise and vibration during the launching and placing of the satellite in orbit and also during the prolonged state of weightlessness while in orbital flight. The study of these problems formed the basis of the biological experiment carried out on the second Soviet artificial earth satellite, which commenced on November 3, 1957.

Preparations for the experiment included the designing of a pressurized capsule in which the animal and the equipment were to be placed, provision of the required environmental conditions and the building of scientific instrumentation for the registration of physiological functions and the hygienic parameters of the ambient medium during the flight. Finally, the experimental animals themselves had to be prepared and trained for the flight.

The pressurized capsule, its equipment and instruments were subjected to numerous tests during prolonged laboratory experiments which led to the improvement of their design and of the reliability of their working. Dogs were selected as experimental animals since they already were used in rocket flights with very good results. The results obtained during the preliminary training experiments indicated that these animals can withstand, quite satisfactorily, prolonged (up to 20 days) confinement in a pressurized capsule of small volume, as well as the effects of vibration, noise and acceleration on laboratory rigs even when exposed to more severe conditions than those which were expected to be encountered during the satellite's flight.

Of the ten experimental animals which underwent complete preliminary training, a dog called Layka, a bitch of about two years old and weighing 6 kg was selected. Layka very quickly and easily became used to the experimental conditions. She was of quiet disposition and showed good resistance to the action of vibration, acceleration and other factors. Directly before the experiment the animal underwent a medical examination in which all the necessary diagnostic methods were used, and was considered to be in a perfectly healthy state and ready for flight.

The equipment of the pressurized capsule was installed and prepared for work: the chemical substance was placed in the regenerating plant; a gelatinous food in the feeding trough; and the whole set of medical apparatus checked. The animal carried sensors for the registration of

various physiological parameters, the sanitary apparel was strapped on at this stage and the animal placed in the capsule (Figure 1).

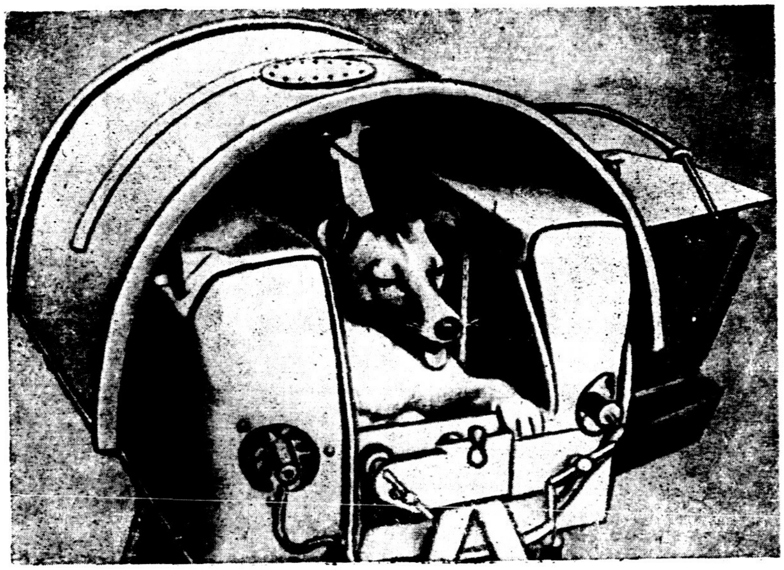

Figure 1. The Dog Layka Before Installation in the Second Artificial Earth Satellite

The capsule together with the animal and all the associated equipment underwent very detailed check for pressurization and was then mounted on the frame of the satellite (Figure 2).

The complex testing of all the apparatus including the telemetering system was satisfactory and therefore the experiment could commence. The separate stages of the experiment differed considerably from each other because different factors acted on the animal at various stages. For that reason, the results of the investigation is best considered according to three basic stages: the pre-launching; the entry of the satellite into orbit; the orbital flight. Pre-launching stage--includes the time from the moment of the placing of the animal in the pressurized capsule to the launching of the satellite. The stay of the animal in the pressurized cabin of the satellite appeared to be fully satisfactory and could not be distinguished from the conditions encountered during prolonged laboratory experiments. During the whole duration of the pre-launching stage, the behavior of the dog Layka and also the indicators of the functional states of the cardiovascular and respiratory systems did not exceed the limits of the usual normal state. The frequency of the cardiac contractions was 78 to 120 beats per minute and the breathing rate 16 to 40 per minute. The average duration of the ECG intervals: R-R, P-Q and Q-T were 0.58, 0.12 and 0.22 seconds respectively.

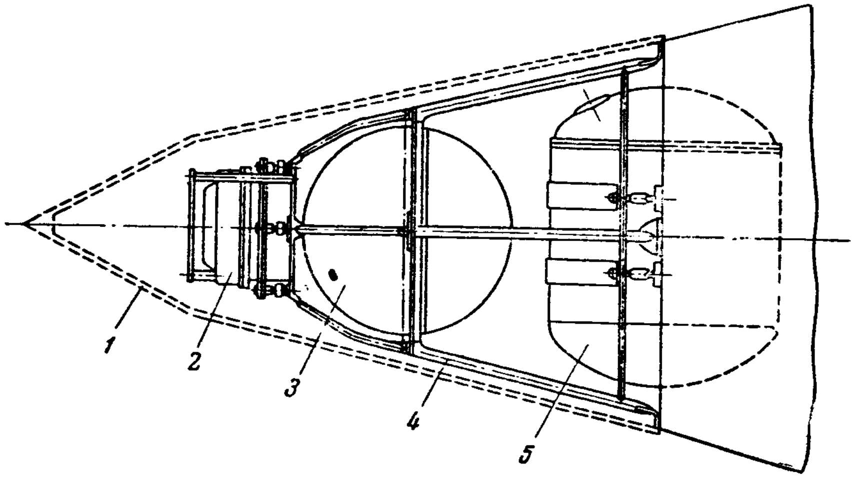


Figure 2. Diagram Showing the Position of the Animal in the Pressurized Capsule in the Second Artificial Earth Satellite

1 - Protective cone, shed after the entry of the satellite into orbit;
2 - Instrument for studying solar ultraviolet and X-ray radiations;
3 - Spherical container with apparatus and radio transmitters;
4 - Carrying frame to which the apparatus is fixed; 5 - Pressurized capsule for the experimental animal

Launching of the satellite on November 3, 1957, necessitated the application of an extremely high velocity which increased rapidly from the time of launching to the entry into orbit. The acceleration with which the satellite was moving exceeded the acceleration due to gravity by a number of times. As a result of this, the weight of the animal's body was increasing. Since the animal was placed perpendicularly with respect to the longitudinal axis of the rocket carrier, the acceleration acted in the direction chest - spine, one of the most comfortable in terms of endurance. Simultaneously with the acceleration the animal was exposed to vibrations and the noise of the engine. The environmental ("hygienic") indicators in the capsule corresponded to the predetermined values.

On the basis of the telemetric information (Figure 3), obtained during this period of the flight, and also from the results of the earlier laboratory tests, it may be said that during the period of the maximal accelerations, the dog was pressed to the floor of the capsule but did not show any noticeable motor disturbance.

Immediately after launching, the frequency of cardiac contractions increased by about a factor of 3. Later on, when the effect of the acceleration was not only continuing but was actually increasing, the

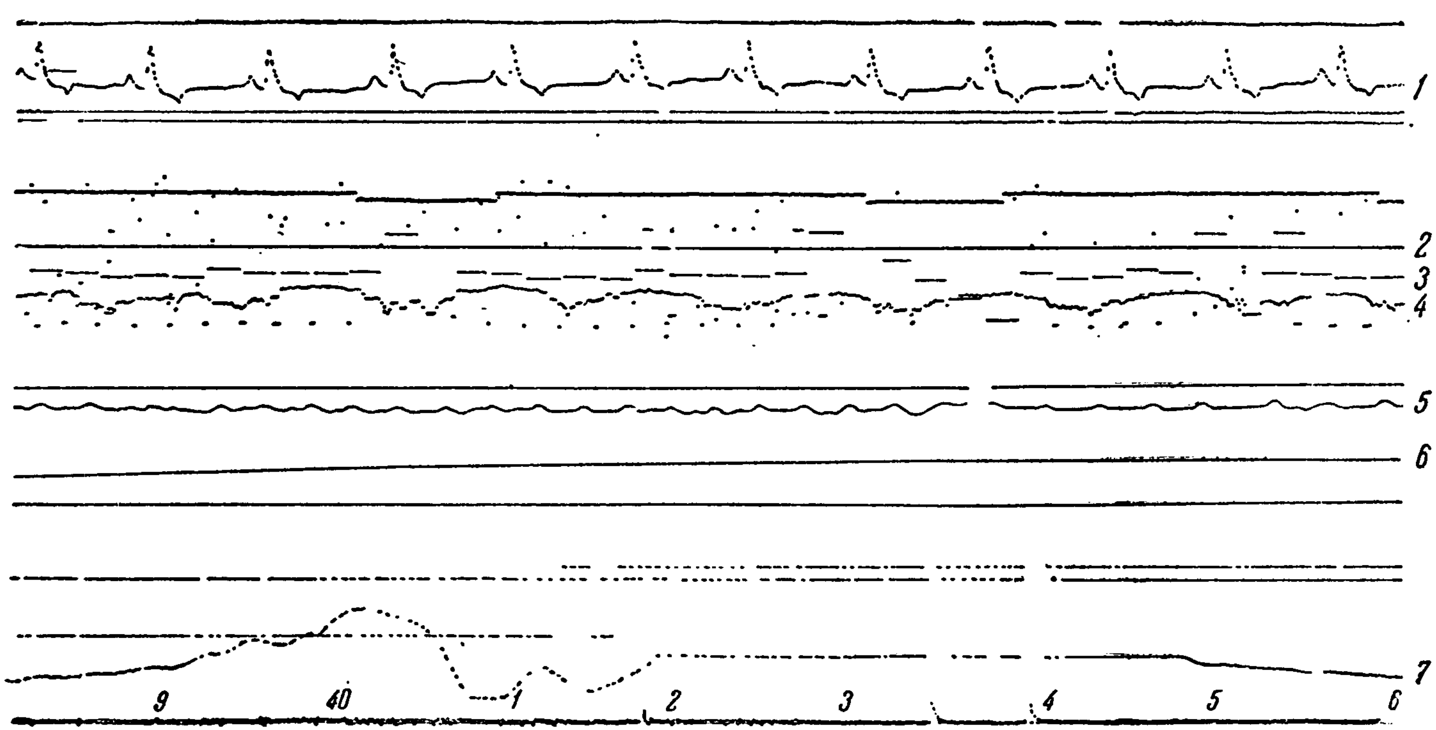


Figure 3. Typical Records Registered during the Satellite's Flight

1 - Electrocardiogram (thorax lead); 2 - Air pressure in the capsule; 3 - Air temperature at various points in the capsule; 4 - Respiration; 5 - Movements of the carotid artery; 6 - Air pressure in the cuff of the sensor measuring arterial pressure; 7 - Movements of the animal (actogram). Numbers along the bottom denote the time scale in seconds.

frequency of the heart beat decreased. No pathological symptoms were found during the analysis of the ECG tracings. Only moderately expressed sinus tachycardia was observed which was clearly of reflex origin. High frequency oscillations were also present on the ECG. These were caused either by the increase in tone or by the contraction of the skeletal muscles (Figure 4).

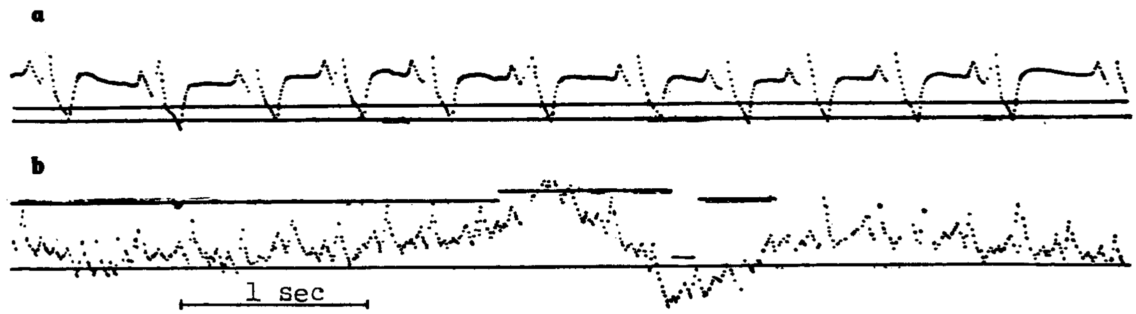


Figure 4. Electrocardiogram of the Dog Layka

A - Before the flight (increased amplification);
B - At the beginning of the powered section of the flight. Changes can be seen in the ECG curve caused by the muscular contractions during the animal's movements.

During the period of maximal acceleration, the rate of respiration exceeded the original value by a factor of 3-4. Mechanical difficulty arising during the movements of the chest was probably chiefly responsible for the increase of the respiration rate under the action of the acceleration.

There are some indications that the changes observed in the physiological functions of the animal during the first moment of the flight were caused by the sudden action of powerful external forces on the organism. The reaction is similar to a type of defensive reflex. Later on, a specific influence of these forces was manifested (e.g., vibration, acceleration). For that reason, the frequency of cardiac contractions, after a certain decrease, continued to be maintained at a higher level than normal. The analysis of the data obtained compared with the results of the earlier laboratory experiments allowed the conclusion to be made that the animal satisfactorily withstood the period of satellite's flight from launching to the entry into orbit. Orbital stage. After the entry of the satellite into orbit, the state of dynamic weightlessness was established during which the weight of the animal was practically nil. In the case of high altitude rockets, the total time of partial and complete weightlessness did not exceed 5 to 6 minutes. During the satellite's

flight, irrespective of its slow rotation, the animal is virtually weightless throughout the whole time. When weightlessness occurs, the body of the animal ceases to press against the floor of the capsule and due to the contraction of the limbs' muscles is easily forced away from it. Judging by the available recordings, the movements were smooth and of short duration. In view of the fact that the thoracic cage was no longer under pressure, the rate of respiration was lowered. After the cessation of acceleration and vibration a very large increase in the heart beat, for a short duration, was observed. The frequency of cardiac contractions continued to decrease and approach the original value (Figure 5).

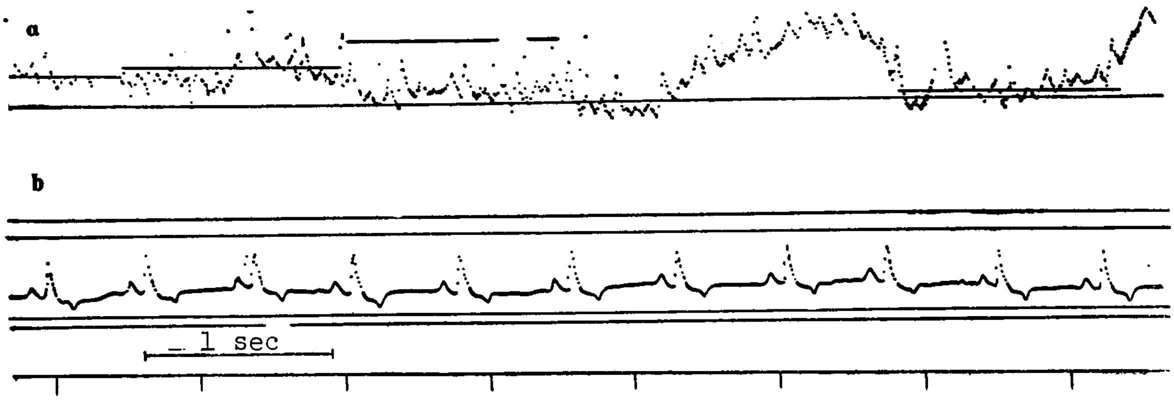


Figure 5. Electrocardiogram of the Dog Layka

A - At the end of the powered section of the flight; B - In the weightless state

When the acceleration ceased, the heart beat returned to the original level at approximately three times slower rate than in laboratory experiments, in which animals were subjected to the action of the same type of accelerations but in a centrifuge. In all probability, this was due to the fact that when accelerations ceased during ground experiments, the animals found themselves in conditions of normal weight. However, during the transition to the orbital motion, the accelerations were changed by the state of dynamic weightlessness. It is possible that the state of weightlessness caused the changes in the afferent signals in the central nervous system (including signals concerning the position of the body of animal in space) and in this way changed the functional state of the sub-cortical regions controlling blood circulation and respiration. As a result of this, a somewhat longer time was required for the functions to return to normal after the influence of accelerations. Obviously, this effect is also somewhat augmented by the presence of other accompanying factors (noise, vibration), the intensity of which during the launching was higher than in laboratory conditions.

We shall now analyze briefly the electrocardiograms taken during the various stages of the satellite's flight (see the Table).

| Time of observation | Interval R-R, sec | | | Frequency of cardiac contractions per min | Interval P-Q, sec | | | Interval Q-T, sec | | | Basset coefficient |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Maximum | Minimum | Average | | Maximum | Minimum | Average | Maximum | Minimum | Average | |
| Before the experiment | 1.35 | 0.35 | 0.58 | 103 | 0.16 | 0.10 | 0.12 | 0.26 | 0.18 | 0.22 | 0.29 |
| At the start of the acceleration | 0.28 | 0.22 | 0.25 | 240 | 0.09 | 0.07 | 0.08 | 0.19 | 0.15 | 0.17 | 0.34 |
| At the end of the acceleration | 0.32 | 0.28 | 0.31 | 190 | 0.10 | 0.10 | 0.10 | 0.20 | 0.19 | 0.19 | 0.34 |
| During the transition to weightlessness | 0.32 | 0.31 | 0.31 | 190 | 0.12 | 0.12 | 0.12 | 0.21 | 0.19 | 0.20 | 0.36 |
| During the state of weightlessness | 0.69 | 0.45 | 0.57 | 103 | 0.12 | 0.09 | 0.10 | 0.25 | 0.21 | 0.22 | 0.29 |

The data given in the table show first of all a considerable increase in the frequency of cardiac contractions at the beginning of the period of acceleration (the interval R-R of the electrocardiogram was decreased to 0.25 sec as against 0.58 sec before the flight). Then the frequency of the cardiac contractions is considerably decreased and during the state of weightlessness corresponds to the original values (interval R-R = 0.57 sec).

It should be noted that the smoothing of the respiratory arrythmia which is very clearly expressed in a dog under ordinary conditions almost disappears during the acceleration. This may be interpreted as being caused by the decrease in the difference between duration of the maximal and minimal values of the R-R intervals. It is possible that the above changes are connected with the lowering of the tone of the vagus nerves, since the injection of atropine during the period of acceleration removes this effect. During the period of weightlessness, respiratory arrythmia returned although it was less severe than before the flight.

During acceleration, the interval R-R (Translator's note: This should be P-Q. The text and table in the Russian do not agree on this point.) tended to decrease (0.08 sec as against 0.12 sec before the experiment); the same was true with respect to the interval Q-T or the time of systole.

Judging by the Basset coefficient, the length of the systolic period was increasing during the active period of the flight. This may be due to the increased functional loading of the cardiovascular system caused by the action of vibrations and accelerations.

It is clear that these changes in the electrocardiogram reflect in principle transient disturbances in the extracardiac heart control and in particular in the predominance of sympathetic (compensatory ?) effects. This is confirmed by the changes in the amplitude of the P wave which increases during the time of acceleration. The variations in the R wave were irregular. The shape and magnitude of the T wave underwent considerable variations. Prior to the launching, the T wave was negative, during the launching the amplitude of the negative T wave increased at first and then started to decrease, while at the beginning of the period of weightlessness this wave became positive. Insofar as the ECG was carried out with a single lead, the interpretation of the above phenomenon is difficult. It may be thought that the changes in the direction and amplitude of the T wave depended on the changes in the position of the heart.

The observed variations in the electrocardiogram were free from any pathological characteristics and were, obviously, due to the increased functional load during the action of accelerations (i.e., during the active stage of the flight). It should be noted that the return to normal of the circulation of the blood and respiration during the period of weightlessness (i.e., orbital flight of the satellite) confirms that this specific factor in itself did not cause any substantial changes in the state of the physiological functions of the animal.

The analysis of the data registering the state of the ambient ("hygienic") parameters in the capsule showed that oxygen was evolving throughout the time of flight in sufficient quantities. The fact that the pressure in the capsule did not drop confirms its reliable pressurization. This is even more important since, as is well known, the satellite passed through regions of meteoric showers. Consequently, the construction of the capsule and its strength gave satisfactory protection against mechanical damage by meteorites.

It was not possible to get a completely clear assessment of the action of cosmic radiation. No clear-cut physiological effects of its action were discovered and a detailed study of this problem will require extensive postflight observations of the animals. This aspect was not catered for under the conditions of the experiment.

The assessment of the results clearly proved that the conditions akin to the cosmic flight are satisfactorily endured by the animal. This positive result derived from the experiment will make it possible to continue and extend the investigations.

It is hoped that the accumulation of experimental data by means of artificial earth satellites will in the near future lead to exact conclusions on problems concerning the medical and biological studies of cosmic flights.

## Conclusions

1. The stage of putting the satellite in orbit was satisfactorily withstood by the experimental animals. The changes noted in the state of the physiological functions were permissible and characteristic of the influence of accelerations, noise and vibrations. Similarities between the respiration and cardiac activity during the active stage of the flight, and the laboratory results in which animals were subjected to the action of the same factors, were observed.

2. The experimental animal satisfactorily withstood the transition from the increased weight (i.e., acceleration) to the state of weightlessness. No harmful effect of weightlessness on the physiological functions of the organisms was observed.

3. During the period of the orbital flight (i.e., the state of dynamic weightlessness) functional indicators of the cardiovascular and the respiratory systems of the experimental animal returned to normal. The animal did not show any noticeable signs of unrest.

## Summary

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The authors review first attempts at biological and physiological investigations in outer space by means of high altitude balloons and rockets and describe the various disadvantages and difficulties encountered in this type of investigation. The main part of this work deals with the experiments carried out on dogs in pressurized capsules of the satellites.

Three main stages of the journey are studied separately, namely, the prelaunching stage, the launching stage and the orbital stage. Electrocardiographic data, air pressure and air temperature data, respiration, carotid artery oscillations, arterial pressure data and actogram data are discussed and tracings of all the above variables shown against a time marker during the launching stage of a satellite.

Further ECG data from the dog Layka at the end of the propulsive stage and during the state of weightlessness are also given, as well as a full analysis of the R-R, P-Q and Q-T intervals at various stages of the flight.

It is concluded that the launching period is satisfactorily endured by the animal and the changes in the physiological function are well tolerated and characteristic of the acceleration, noise and vibrations. Full analogy between the respiratory and cardiac activities encountered during the propulsive stage of the flight and those in simulated laboratory tests was established. The animals endured equally well the transition from increased weight to the state of complete weightlessness.

Finally, during the orbiting state, a considerable normalization of the cardiovascular and respiratory systems was observed. There was absence of any motor disturbance during that stage.

A rough diagram showing the disposition of the pressurized capsule in the cone of the carrier rocket, together with other equipment, i.e., UV and X-ray detectors and radio transmitters, is also given. Author

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# PART IV

## GROUND EXPERIMENTS, METHODS AND TECHNIQUES

### THE AMINOACID COMPOSITION OF CHLORELLA

### Chlorella pyrenoidosa

N. M. Sisakyan, E. N. Bezinger and M. G. Shaposhnikova

One of the more important problems in preparing for long space flights is the provision of the crew with all requirements for normal life--air, food, water, etc.

Simple calculations indicate that for prolonged flights astronomical quantities of food and oxygen would have to be carried, making the flight quite impracticable.

A considerable advantage can be achieved by establishing a "water cycle" in the capsule. The water consumed by the crew is completely excreted. All the excretions can be collected and purified by special equipment so that the "regenerated water" will be suitable for reuse.

Methods of regenerating the oxygen required for breathing have also been sought. Three basic methods of regeneration have been established: chemical, physicochemical and biological.

The last has a number of obvious advantages. Using photosynthesis, green plants placed in the capsule of a spacecraft would absorb carbon dioxide from the atmosphere and give off oxygen in the same way as they do on the Earth. At the same time synthesis of organic compounds which form part of the new growth of the plant will take place. Two important advantages are evident: the atmosphere becomes enriched with oxygen, and there is synthesis of food substances suitable for animals and man.

Some plants, for instance the single-celled alga Chlorella, have a particularly high rate of oxygen production. It has attracted the attention of scientists and serves as the subject of many investigations.

Animals, together with plants, form on board the spacecraft an artificial ecological medium which would provide food and oxygen for man.

Essentially this would mean that inside the capsule a closed metabolic cycle would be created, similar to that which exists on Earth.

The only open link in this scheme is the energy balance. This has to be supplied from the outside, stored and suitably transformed. The energy source in space is the same as on Earth, namely, the Sun.

During the growth of algae, a product is obtained which consists almost entirely of organic substances that are easily assimilated by animals. It is of particular importance that the chemical composition of the algae depends to a very large extent on the conditions of cultivation (Fisher and Burlew, 1953; Spoer and Milner, 1949) and therefore can be controlled. Thus, the protein content can vary between 8.7 and 58 percent (Milner, 1953), depending on the amount of nitrogen available to the plant (Fisher, 1953).

Among plant materials, Chlorella has a particularly high protein content, namely, about 50 percent of its dry weight, which is considerable higher than that of the best plants used as food (Fisher and Burlew, 1953).

This enables one to understand the great interest manifested towards the large scale cultivation of algae, as a potentially inexhaustible new mass source of proteins, fats, vitamins and other substances of nutritional, dietary or technical significance.

Protein plays an important part in the nutrition of man and animals. However, proteins differ as regards their food value.

Obviously, the unequal nutritional value of different proteins is due to their differing content of special, so-called essential, aminoacids which cannot be replaced, which are necessary for the body and which cannot be synthesized by animals. At present it has been established that eight aminoacids are essential for the human being; tryptophan, phenylalanine, methionine, lysine, valine, threonine, isoleucine, and leucine. With regard to arginine and histidine, the literature data are controversial.

In order to obtain correct data on the aminoacid composition and subsequently on the dietary value of a product, it is necessary to study the whole complex of proteins contained in it.

The object of the present investigation was an intensive culture of Chlorella pyrenoidosa grown in an inorganic medium (material supplied by the photosynthesis laboratory of the K. A. Timiryazov Institute of Plant Physiology).

A given quantity of freeze-dried Chlorella was hydrolyzed for 24 hours at 105° C with 200 parts by weight of 6N HCl. By this method of hydrolysis very little humin formation occurs, and consequently there are

also low losses of aminoacids on analysis even in the presence of a large amount of carbohydrate. This method of hydrolysis was developed for food products of plant origin (Schram, Dustin et al., 1953; Dustin et al., 1953). The advantages offered by this method were confirmed by Yu. B. Filipovich (1958) in the analysis of oak leaves and preparations of whole caterpillars and larvae of the oak silkspinner, and were frequently observed by the authors in the hydrolysis of protein preparations.

After evaporation of the hydrolysate in vacuo, the excess of hydrochloric acid was removed by repeated washing of the dry residue followed by evaporation in a vacuum desiccator over KOH. The dry residue of aminoacids was redissolved and a 0.38 to 0.43 percent solution was made, which was studied by chromatography in a mixture of butanol-acetic acid-water in the ratio of 4:1:5, and by the method of McFarren (1951) in orthocresol and phenol. Separation of the aminoacids was obtained by a repeated passage of solvent through the chromatogram. For the quantitative determination of the aminoacids the method of Lissitzky and Laurent (1955) modified by Kretovich and Uspenska (1958) was used. The calculation of the percentage content of aminoacids in the Chlorella hydrolysate was carried out by comparison with the amount of aminoacids contained in a standard mixture which was hydrolyzed and analyzed chromatographically under the same conditions as the Chlorella preparation.

The determination of the total nitrogen content of Chlorella was carried out by the Kjeldahl method. The same method was used for the determination of protein nitrogen after precipitating the proteins with 5 percent trichloroacetic acid. The total nitrogen content was 5.6 percent, protein nitrogen 4.3 percent, and the protein content of the preparation 26.9 percent (based on dry weight). The table gives the results of the aminoacid analyses carried out on the Chlorella hydrolysate. The values obtained by paper chromatography are the average values of 6-12 determinations.

The table also includes for comparison data on the aminoacid composition (Fowden, 1952) of the protein fraction of Chlorella vulgaris and the aminoacid composition of the most valuable protein from the dietary point of view, namely casein (the data for casein were taken from the book by R. Block and D. Bolling, "The Aminoacid Composition of Proteins and Foods", 1949). The protein of Chlorella contains all the aminoacids essential for man. In our work tryptophan was not determined, but this aminoacid, according to the data of other investigators, is contained in the proteins of Chlorella (Fowden, 1951, 1952, 1952a). In its content of essential aminoacids, Chlorella is close to casein. It contains the same amounts of lysine and threonine, and somewhat smaller amounts of phenylalanine and valine. Chlorella is poorer in leucine and isoleucine, and considerably poorer in glutamic acid. The relatively low methionine content is noteworthy. On the other hand, Chlorella contains larger amounts of the basic aminoacids (arginine and histidine) which play an important part

Aminoacid Composition of Chlorella

| Aminoacid | Hydrolysate of Chlorella pyrenoidosa (experimental data) | | Protein fraction of Chlorella vulgaris (Fowden) | Casein (Block and Bolling) |
|---|---|---|---|---|
| | g per 100 g Chlorella | g N aminoacid per 100 g protein N | | Per 16% N |
| Aspartic acid | 2.7 | 6.5 | 6.4 | 6.3 |
| Glutamic acid | 2.9 | 6.3 | 7.8 | 22.8 |
| Serine | 1.7 | 5.9 | 3.3 | 6.7 |
| Threonine | 1.3 | 3.6 | 2.9 | 3.9 |
| Glycine | 1.5 | 6.6 | 6.2 | 0.5 |
| Alanine | 2.4 | 8.8 | 7.7 | 5.6 |
| Valine | 1.7 | 4.8 | 5.5 | 7.0 |
| Leucine | 3.2 | 8.0 | 6.1 | 12.1 |
| Isoleucine | | | 3.5 | 6.5 |
| Phenylalanine | 1.6 | 3.1 | 2.8 | 5.2 |
| Tyrosine | 2.1 | 3.6 | 2.8 | 6.4 |
| Methionine | 1.1 | 2.2 | 1.4 | 3.5 |
| Arginine | 1.6 | 11.6 | 15.8 | 4.1 |
| Histidine | 0.8 | 4.8 | 3.3 | 2.5 |
| Lysine | 1.6 | 7.0 | 10.2 | 6.9 |

in nitrogen metabolism and may satisfy the requirements of the body with regard to these aminoacids and also enter into various synthetic processes.

It was found that some of the aminoacids (aspartic acid, glutamic acid, alanine, proline, valine, tyrosine, threonine, serine, lysine and leucine) are contained not only in the bound but also in the free state, which agrees with the results of other authors (Stepka, Benston and Calvin, 1948). Certain differences in the values obtained by Fowden, who studied the aminoacids of Chlorella only in the protein fraction, may be explained by the presence of these free aminoacids. The aminoacid content of proteins may vary considerably according to age, changes in nutritional conditions, and varying degree of illumination (Osipova, 1953; 1960; Kizel' et al., 1936; Sisakyan, Bezinger and Gumelevskaya, 1953; Sisakyan, Bezinger, Gumelevskaya and Luk'yanova, 1955). The effect of these factors may also explain the discrepancy between our figures and the data of other authors. Fisher and Burlew (1953) quote an aminoacid composition of Chlorella proteins which indicates a high quality protein, the main insufficiency of which is the low content of methionine.

The investigations of Fowden (1951, 1952, 1952a) on the aminoacid composition of Chlorella proteins prove that it is possible to use cultivated algae as a source of dietary, nutritional and industrial raw material.

Only isolated investigations have been published so far which attempt to show the suitability of Chlorella for the above aims. For instance, it may be mentioned (Fisher and Burlew, 1953) that an experiment was carried out on feeding rats on Chlorella, which did not, however, give definite results. It should be noted that rats which received additional methionine grew well. In similar tests carried out in England (Geoghegan, 1953) the ration of young rats was enriched with 17 percent of dry preparations of Chlorella and their protein efficiency determined, i.e., the increase in bodyweight in grams per gram of protein. This diet did not produce any harmful effects in the animals.

In the U.S.A. in 1951, an experiment was carried out in which dry Chlorella was given to chicks fed on a qualitatively deficient diet. The growth improved, which could be ascribed to the action of the carotin complex of Chlorella (Wiensberg, 1957). More recently, the utilization of mass cultivation of algae becomes of considerable importance in national agriculture as a source of additional protein-vitamin food for man.

The achievements in the mastery of space have attracted attention to the problem of cultivating unicellular algae for the biological regeneration of the air, and simultaneous acquisition of an additional source of food, as well as the utilization of human excreta in pressurized capsules (Resolution of the All-Union Conference on the Cultivation of Unicellular Algae, 1961).

As practical parts of the possibility of utilizing Chlorella for human nutrition, one may refer to the case of the Japanese nation in which marine algae are widely used for food, and to the preparation and consumption of various dishes with a considerable addition of Chlorella which have an acceptable taste (Fisher and Burlew, 1953; Morimura and Tamiya, 1958).

Summary

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The aminoacid composition of Chlorella pyrenoidosa, grown in an intensive culture, was investigated. Published information and the results of the here described experiments prove that this plant contains all the aminoacids required by man in quantities close to those of casein, which is a protein of high nutritive value.

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# THE EFFECT OF GENERAL VIBRATION ON THE ANIMAL BODY

V. S. Georgiyevskiy and Ye. M. Yuganov

The action of the carrier rocket of an artificial earth satellite is accompanied not only by acceleration and engine noise, but also by high frequency vibration which may reach very significant magnitude.

A considerable amount of work has been devoted to the problem of vibration and its effect on cardiovascular and respiratory function; however, in the majority of cases, the data relate to the effects of local vibration only. The investigations have shown that the chronic effect of local vibration with frequencies higher than 50 cycles causes vasoconstriction and an increase in arterial pressure; on the other hand, the effect of vibration of frequency less than 50 cycles caused vasodilatation and a fall in arterial pressure (Brodnitz and Wollheim, 1931; Vol'fovskaya, 1932; Vegger and Vasilenko, 1934; Butkovskaya, 1952; Andreyeva-Galanina, 1956; Agashin, 1954).

The physiological effect of low frequency vibration is somewhat different when exerted on a large surface of the body (Agashin, 1957). In this case the arterial pressure is increased. The pulse rate is also increased and certain changes in the venous pressure occur (Butkovskaya and Agashin, 1954; Genitsinskiy, 1926).

The effect of high frequency vibration on animals and human needs further study. In the existing literature there are certain contradictions. Certain investigators consider that the action of vibration on all or a large part of the body causes in man a rise in arterial pressure and heart rate (Coermann, 1940; Borshchevskiy et al, 1958); others claim that there is a possibility of completely opposite changes taking place (Agashin, 1957).

These and many other data were obtained from experiments in which the effect of vertical vibrations on man was studied. The effect on animals of vibrations acting in a direction perpendicular to the longitudinal axis of the body has not so far been studied. As is well known, the reaction of the body to vibration depends not only on its physical parameters but also on the direction in which the vibration acts (Reiher and Meister, 1937).

The object of this investigation was to study the main physiological functions of animals when they were exposed to general vibration acting in a transverse direction to the longitudinal axis of the body.

The investigations were conducted in the laboratory on a VP-70 vibration stand capable of vibrating at frequencies from 10 to 70 cycles with an amplitude of 0.4 to 2.5 mm. In planning the experimental techniques allowances were made for the fact that during the period of propulsive movement of the satellite high frequency vibrations are predominant. The above conditions, and also the capabilities of the vibrating stand, determined the choice of physical parameters of vibration which were employed in this experiment. Thus, the investigations were carried out under the action of vibration of 70 cycles and an amplitude of 0.4 mm, which corresponds to a vibration acceleration of 7.7 g. The duration of action was 6 minutes. This was a sufficient period to determine the physiological effect of a single exposure to vibration, since, according to the data of a number of authors, well defined changes in physiological functions occur within the first five minutes.

The arterial pressure, pulse and respiration rates were recorded by means of various aviation medical devices before, during and after the experiment.

Arterial pressure was measured by the oscillatory method, the heart rate from the oscillation count and the frequency of the bioelectric systems in the ECG recording. The registration of the respiration rate and the magnitude of the arterial pressure commenced a minute before the beginning of vibration and was continued for a minute after the vibration had ceased. The recording of the heart potentials was carried out in separate cycles but extending for the same periods of time. In addition, all these functions were recorded 3, 5, 10 and 15 minutes after completion of the period of vibration.

The investigations were carried out in two stages. In the first, the orientational reaction of the animal to the surroundings (presence in the room, attachment to the cradle, the effect of the noise of the vibration stand, etc.) were all extinguished. In the second stage the direct effect of vibration was studied. The studies were carried out on seven animals. Altogether, in both stages, 61 experiments were carried out.

The first stage of the work consisted of training the animals, which were placed above the stand in such a way that they were not subjected to vibration but only to the effect of the noise of the vibration stand motors, which was of an intensity of 85-90 decibels. In all these experiments the indicators of the physiological functions of the animals were periodically registered to determine the effectiveness of the training in comparison with the data obtained during the action of vibration. The training period was completed when the physiological changes were insignificant and the general level of the functional parameters did not differ substantially from the original. During the first stage 54 experiments were carried out altogether at intervals of 1-3 days. The amount of training depended on the individual peculiarities of the animals and

on the type of their higher nervous activity. In certain animals training was sufficient after only 5 or 6 tests; others required as many as 12 to 14 training experiments. Apparently the first group belonged to the inhibited and the second to the excitable type.

As a result of this training the dogs lost their orientating and defensive reflexes to the surroundings. The animals ran into the laboratory without any compulsion, the noise of the motor of the vibration stand did not cause in them any essential changes in physiological functions, etc.

Data illustrating the changes in the dog Kozyavka in the pulse and respiration rate and also the maximum arterial pressure under the influence of the noise of the vibration stand motors before and after training are shown in Table 1. Before training the physiological functions in animals exposed to the effect of noise changed quite significantly. The respiration rate was increased almost twice, the cardiac contractions 1.5 times and the maximum arterial pressure by 20 mm Hg. After completion of the training experiments the physiological functions in the presence of noise did not change appreciably beyond the usual fluctuations. Similar data were also obtained when training other animals.

In the investigations of the second stage the animals were subjected to the combined effect of noise and vibration. For this purpose the dog was placed on the cradle, which was rigidly secured on the platform of the vibration stand.

Seven experiments were carried out one by one on each animal. The behavior of all the animals and the nature and degree of changes in the physiological functions were identical.

Thus, the state of the dog Layka during the whole period of vibration remained satisfactory. Its respiration was regular and deep and its general behavior calm. Only at the time when the vibration stand was turned on did the dog attempt to get up, but it immediately calmed down and lay down in its cradle throughout the entire experiment. After the experiment no peculiarities in the behavior and condition of the animal were noticed (Table 2, Figure 1).

From the data shown in Table 2 it is clear that the heart rate during the period of vibration increased to 64-96 per minute (almost twice) and immediately after the vibration it decreased although it did not reach the original level. The maximum arterial pressure increased insignificantly (by 30 mm Hg), and decreased after completion of the experiment, reaching the original figure. On analysis of the ECG a decrease in the R - R interval was noted during the vibration period, which corresponded to the increase in the heart rate. No characteristic changes were observed in the respiration rate.

Table 1. Changes in Physiological Functions of the Dog Kozyavka Under the Influence of Noise

| Function | Time of investigation | Before exposure to noise | During exposure to noise | | | | | | After exposure to noise | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1' | 2' | 3' | 4' | 5' | 6' | 1' | 3' | 5' | 10' | 15' |
| Respiration rate per minute | Before training | 74 | 67 | 77 | 86 | 116 | 135 | 137 | 326 | 100 | 104 | 90 | 80 |
| | After | 30 | 28 | 35 | 36 | 37 | 33 | 35 | 41 | 38 | 40 | - | - |
| Pulse rate per minute | Before training | 138 | 198 | 216 | 213 | 198 | 188 | 195 | 195 | 172 | 164 | 152 | 144 |
| | After | 120 | 138 | 123 | 126 | 126 | 122 | 123 | 123 | 126 | - | - | - |
| Maximal arterial pressure, mm Hg. | Before training | 180 | 190 | 200 | 195 | 200 | 195 | 190 | 200 | 190 | - | - | - |
| | After | 185 | 180 | 180 | 190 | 180 | 180 | 180 | 170 | 180 | 180 | 160 | - |

Table 2. Changes in the Physiological Functions of the Dog Layka Under the Influence of Vibration

| Function | Before vibration | Period of vibration | | | | | | Period immediately after vibration |
|---|---|---|---|---|---|---|---|---|
| | | 1' | 2' | 3' | 4' | 5' | 6' | |
| Respiration rate per minute | 36 | 40 | 28 | 24 | 39 | 34 | 32 | 39 |
| Pulse rate per minute | 106 | 170 | 176 | 185 | 180 | 180 | 202 | 136 |
| Maximal arterial pressure, mm Hg. | 140 | 150 | 150 | 160 | 160 | 160 | 170 | 140 |

The behavior of the dog Kozyavka during the experiments was calm and its respiration was regular and deep. Towards the end of the experiment, as a result of an accidental stimulus, its respiration rate increased significantly (Table 3, Figure 2).

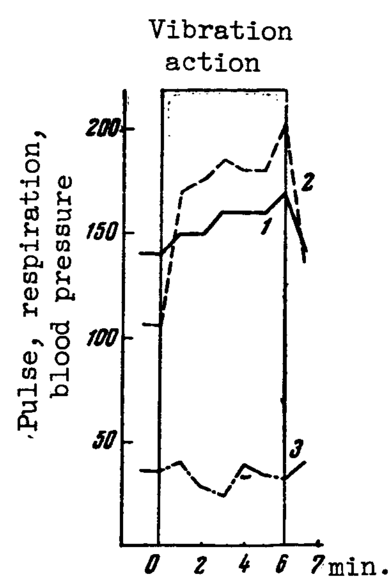


Figure 1. Change in the maximum arterial pressure (1), pulse rate (2) and respiration rate (3) in the dog Layka when exposed to vibration

Table 3. Effect of Vibration of the Physiological Functions of the Dog Kozyavka

| Function | Before vibration | Period of vibration | | | | | | After vibration | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1' | 2' | 3' | 4' | 5' | 6' | 1' | 3' | 5' | 10' |
| Respiration rate per minute | 17 | 25 | 33 | 31 | 28 | 110 | 74 | 26 | 26 | 16 | - |
| Pulse rate per minute | 126 | 189 | 183 | 180 | 171 | 170 | 171 | 144 | 135 | 129 | 110 |
| Maximal arterial pressure, mm Hg. | 135 | 195 | 200 | 200 | 195 | 190 | 190 | 150 | 140 | 135 | 135 |

The second series of investigations showed that the animals satisfactorily endure the 6 minutes of transverse vibrations of 70 cycles frequency at an amplitude of 0.4 mm (vibrations acceleration 7.7 g). No noticeable deviation in the behavior and general condition of the dogs was detected. The effects of vibration caused a considerable increase in the heart rate (almost twice) and a moderate increase in maximum arterial pressure (30-65 mm Hg), which returned to its original level 5 minutes after completion of the action of vibration.

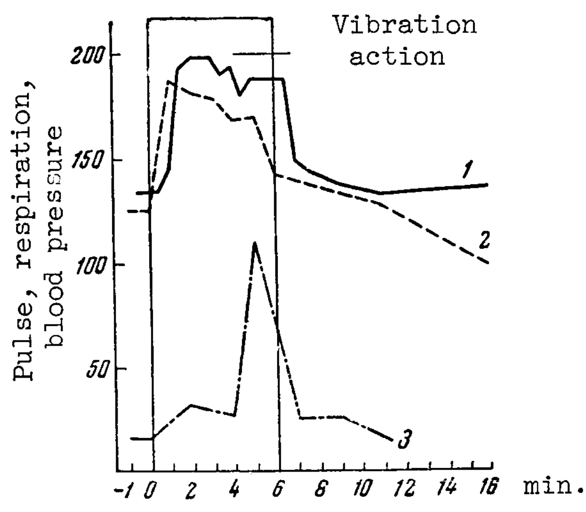


Figure 2. Change in the maximum arterial pressure (1), pulse rate (2) and respiration rate (3) in the dog Kozyavka when exposed to vibration

Comparing the isolated effect of noise in the first training experiment with the effect of vibration, it may be seen that the maximum arterial pressure in the animals increases. However, in the first case it increases altogether by 15-20, and in the second case by 30-65 mm Hg.

The experimental results obtained permit estimation of the specific significance of vibration in the changes in the cardiovascular and respiratory functions of the animal during the propulsive part of satellite flight.

The results of the investigations and the literature data indicate that the nature of the change in the heart rate and arterial pressure of animals during exposure to vibration directed transversely to the body axis does not differ substantially from the changes in these functions in man during vertical vibration. The general level of the animal reaction exceeds noticeably that of the similar reaction in man. In the short term exposure to vibrations the animals did not show any signs of adaptation which, according to the data given by A. I. Vozhzhova (1947), is characteristic for human beings.

The above investigations, in addition to their immediate utilitarian value, have also other independent values. Although they do not pretend to give the final solution of the problems discussed, they nevertheless may serve as good background material in studying the problem of the effect of "transverse" vibration. Subsequent work should proceed along the lines of accumulation of more factual material and the study of mechanisms occurring during the changes in physiological functions under the influence of vibration. At present there are only relatively few works available dealing with the subject (Agashin, 1957; Ryumin, 1950; Antonovskiy and Krichevskiy, 1929; Vaynshteyn, 1937; Gratsinskaya, 1949); thus at present there is no complete integral theory explaining fully the essence of the physiological mechanism of vibration.

## Conclusions

1. Animals (dogs) endure satisfactorily the six-minute effect of general vibration of 70 cycles frequency at an amplitude of 0.4 mm (vibration acceleration 7.7 g), acting in transverse direction to the longitudinal axis of the body.

2. The action of vibration on the above parameters causes in animals an increase (approximately twice) in the heart rate and a moderate increase in arterial pressure.

Summary

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Responses of some physiological functions to general vibrations have been investigated to elucidate the action of vibrations appearing at the active phase of a satellite flight upon the animal organism.

Vibrations caused a considerable increase in the rate of heart muscle contractions and a moderate rise in arterial blood pressure. The changes in physiological functions observed can be of help in analyzing the effect of the flight on the animal on board the satellite. Author

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# THE EFFECT OF PROLONGED TRANSVERSE ACCELERATION ON ANIMALS

A. R. Kotovskaya and Ye. M. Yuganov

High speed flights in jet-propelled vehicles make it necessary to study the physiological influence of prolonged periods of acceleration on animals and human beings. Numerous investigations have shown that the living body best withstands transverse accelerations.

A large number of published works on this subject are devoted to changes in physiological functions and to determination of limits of endurance of accelerations taking place during catapulting and crash landings of aircraft (Armstrong, 1952; Bergin, 1949; Gauer Ruff, 1933; Stapp, 1955; Isakov, 1957; Gozulov, 1957; Tsvetkov, 1957).

According to Buhrlen (1938), the effect of a transverse acceleration of 4 g causes in a man certain difficulties in breathing, which at an acceleration of 10 g was significant and at the level of 14-15 g appeared almost unbearable. Studying the changes in breathing during accelerations of 5-7.8 g acting in the direction back to front and front to back, D. V. Sobol' (1947) observed, together with difficulty in breathing, pallor or reddening of the facial skin.

According to Armstrong (1952) and Bedzhina (1949), the effects of transverse accelerations exceeding 6-8 g caused disturbance and 10 g caused difficulty in breathing. Gauer (1938) showed that during accelerations in excess of 2 g the quickening of respiration is accompanied by increased pulmonary ventilation. Difficulty in breathing and also sinus tachycardia were also observed by Gell and Khanter (1954). Increased pulse rate was also noted by Lambert (1945) during accelerations up to 10 g acting for 3-5 seconds in the direction of back to front. At the same time Beckmann, Duance, Ziegler and Hunter (1955), who subjected experimental animals to the effects of transverse accelerations of 3 (2 seconds) to 15 g (5 seconds), did not notice any differences in the heart rate before and after the experiment.

In all the above enumerated investigations the action of the acceleration was of short duration. Only isolated studies were concerned with the prolonged effects of transverse accelerations. Ballinger (1952) subjected his experimental human beings when lying on their backs to the action of accelerations from 3 (9 minutes) to 10 g (2 minutes 6 seconds) and observed difficulty in breathing, shortness of breath, vertigo and pain in the retrosternal and epigastric regions. The general condition

of the subjects tested was estimated only from their interrogation concerning health. Physiological functions were not registered, so that it was not possible to make any definite judgment concerning the state of respiration and blood circulation during exposure to prolonged acceleration.

Several papers were presented at the Conference on Aviation Medicine in Stockholm (1957), devoted to the prolonged action of transverse acceleration on the human body. All the information contained in these papers points to the need for using special anti-gravity suits (of the altitude-compensating type) in order to protect the body from the injurious influence of prolonged transverse accelerations. Using these protective devices, human beings were able to withstand satisfactorily accelerations of the order of 2 g for a period of 30 minutes, and of 3 g for a period of 6-7 minutes (Clarke et al., 1957); it was also possible to protect them from vascular injury (Cunningham et al., 1957).

The above investigations were all concerned with the effects of prolonged acceleration of constant magnitude. The literature contains very little information on the effect of prolonged transverse acceleration of varying magnitude on the body. At the same time this problem is now becoming urgent in connection with the need of securing flights of living organisms in high-altitude rockets and artificial satellites.

The purpose of the present investigation was to determine the effect of prolonged accelerations of variable magnitude on living organisms, the accelerations acting in the transverse direction to the axis of the body. Experiments were carried out on 14 dogs. The animals were placed on a centrifuge and subjected to centripetal accelerations up to 10 g in the direction of front to back. One hundred and five experiments of different durations were carried out, viz: 3, 6 and 15 minutes, of which 40 tests lasted for 6 minutes (20 of these were training and 20 the main runs).

There are further quoted the results of 6-minute exposure of the animals to accelerations.

To assess the state of respiration and blood circulation, the following parameters were used: the heart rate determined by the electrocardiogram (ECG); the respiratory rate determined from the changes in the perimeter of the chest; maximum blood pressure recorded by the oscillator method during periodic constriction of the carotid artery lifted into a skin flap.

The last mentioned parameters were recorded on a KAMA apparatus before and during the action of the acceleration and also 5, 10 and 20 minutes after its termination. Certain auxiliary observations were also carried out during this experiment, namely the behavior of the animal and

its reaction to surrounding situations were also noted. During training experiments, the animals were trained to become accustomed to the location of the experiment, to the various sensors, to their harness, etc.

The results of the experiments have shown that the behavior of the animals during the action of the accelerations was relatively calm. At the beginning of the rotation on the centrifuge the animals appeared to have an orientating reaction with phenomena of motor excitation. As the magnitude of acceleration was increased, some animals remained calm while others developed a noticeable motor disturbance.

Only to a definite value of acceleration did all the animals retain their resistance to the increase of body weight and retain freedom of movement of head and trunk. Then they appeared to be pressed to the surface of the harnessing attachment (cradle) without developing any noticeable motor disturbances. During rotation, the animals suffered a marked increase of salivation, which continued for a certain time after rotation had ceased.

The heart rate from the beginning of the action of acceleration increased rapidly and exceeded the original frequency by 1.5-2 times; with certain variations it persisted at this level during the entire period of acceleration (Figure 1). After acceleration ceased, there was a gradual reduction in the heart rate which returned to the original level within approximately 5-10 minutes.

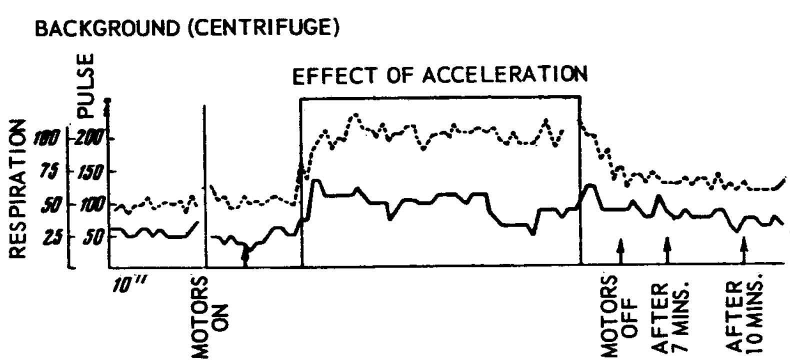


Figure 1. Change in pulse rate (1) and respiration rate (2) during the action of acceleration varying from 2 to 10 g over a period of 6 minutes (dog Polkan, experiment 1, 30 May 1957)

The usually well defined respiratory arrhythmia either disappeared completely or became less significant during the acceleration (see Figure 2). At the beginning of rotation the respiration rate as a rule increased by 1.5-2 times as compared with the original rate. As the magnitude of acceleration increased, the body of the animal was pressed more strongly

on to the surface of the cradle, which resulted in hampering of the respiratory movements; the respiration also become more shallow and frequent. Throughout the whole period of acceleration the respiration rate exceeded the original value by 1.5-3 times. When the acceleration ceased, the respiratory movements slowed down and returned to their original level within about 5 minutes (Figure 2).

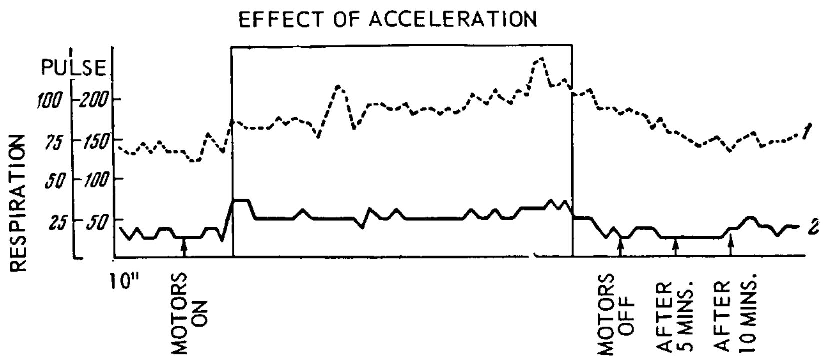


Figure 2. Oscillograms of pulse (1) and respiration (2) during the action of acceleration from 2 to 10 g over a period of 6 minutes (dog Umnitsa, experiment 2, 11 July 1957)

The magnitude of the maximum arterial pressure at the beginning of acceleration increased when compared with the original by 50-80 mm Hg and persisted at that level throughout the whole period of acceleration. When the rotation ceased there was a marked gradual lowering of arterial pressure until within about 5-10 minutes it returned to the original value.

Surprisingly enough, in certain animals during repeated experiments (starting with the second experiment), immediate reactions in the blood circulation and breathing were observed, which started immediately after switching on the centrifuge motor and developed even before triggering the effect of acceleration could be felt by the animal. Thus, the noise of the installation acted as a triggering signal.

During the action of acceleration the behavior of the various experimental animals and the changes in heart and respiration rates were sufficiently uniform, so that below we quote only the data of two experiments in this particular series.

Dog Layka, a bitch, between 1.5 and 2 years old, weight 6 kg, of very equable disposition, accustomed herself very well and very quickly to the experimental conditions. In experiment 2, of 17 October 1957, the behavior of this animal during the entire period of acceleration and afterwards remained calm; in the course of the first 5 minutes the dog

retained freedom of movement of head and trunk. Then, with increasing acceleration, Layka appeared to be pressed to the surface of the cradle and did not develop noticeable motor disturbance.

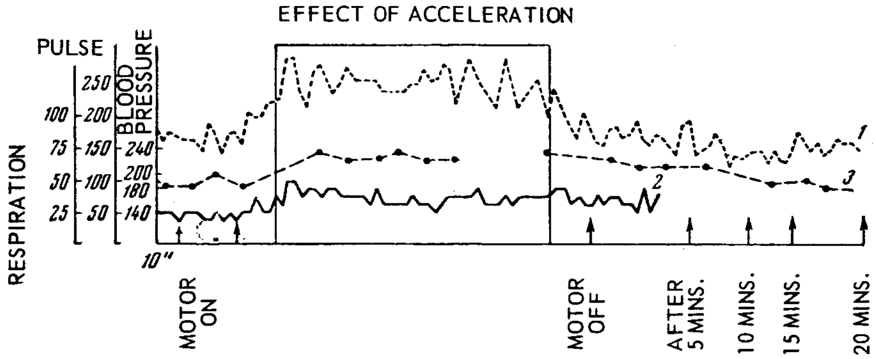


Figure 3. Change in pulse rate (1), respiration (2) and blood pressure (3) during the action of acceleration from 2 to 10 g for a period of 6 minutes (dog Layka, experiment 2, 17 October 1957)

As is shown in Figure 3, the heart rate and maximum arterial pressure changed immediately after the centrifuge was switched on. The pulse rate increased to 200 beats per minute from the moment acceleration began (against 140-150 before the motor was switched on), and with start of acceleration it rapidly increased further to 290 and was maintained within the range of 220-290 throughout the whole period of the action of acceleration. After the acceleration ceased, the pulse rate fell and the original level was established within 5 to 10 minutes. The respiration rate, from the moment of switching on the motor, increased up to 40-50 per minute (against the original 20-25) and reached 53 at the beginning of the rotation. Later on, the frequency of respiration varied within the limits of 30-35 per minute.

The maximum arterial pressure at the beginning of rotation increased to 210-230 mm Hg (against 180-190 in the original state) and was maintained at this level throughout the whole period of acceleration. When the rotation ended, the pressure gradually decreased and approached the original level after 10 minutes.

The dog nicknamed Umnitsa, a bitch, 3-4 years old, weighing about 7 kg, was a lively animal, calm in working situations, which quickly and easily became familiar with the conditions of the experiment. In experiments carried out on 11 July 1957, the behavior of the animal throughout the acceleration and afterwards was very calm. During the first 4 minutes of acceleration the dog retained freedom of movement of head and

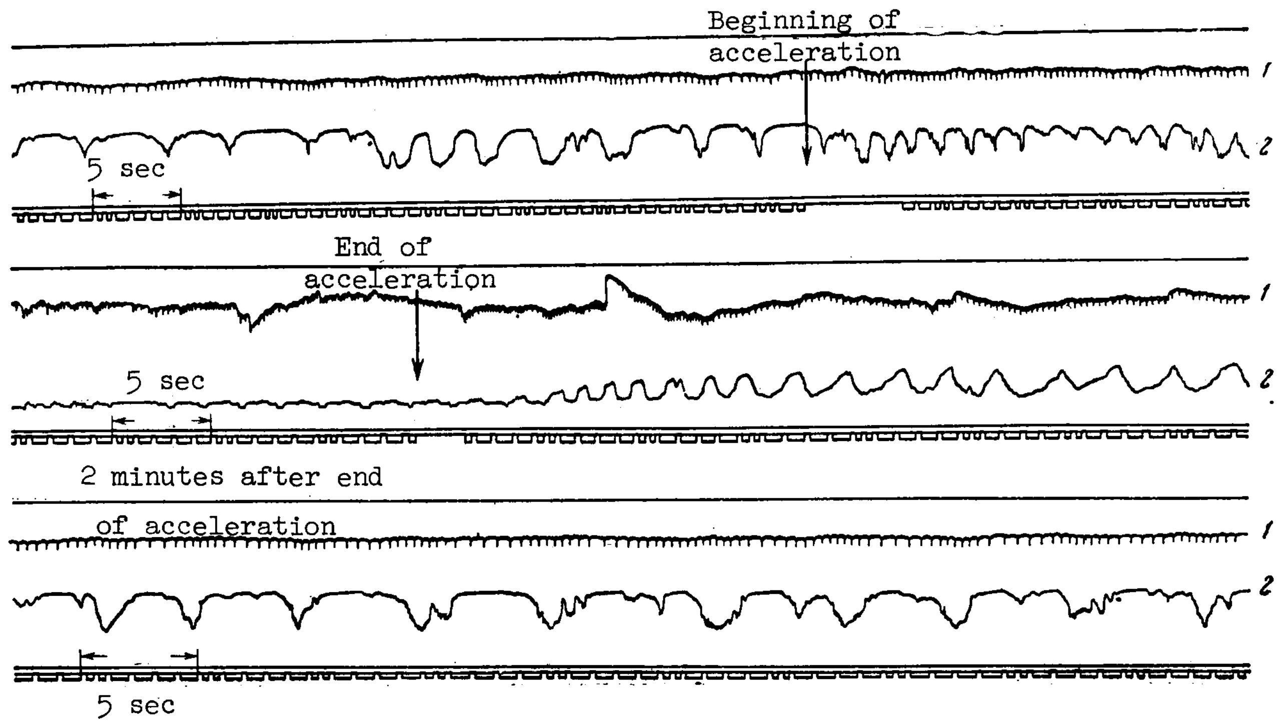


Figure 4. Change of pulse rate (1) and respiration (2) during the action of acceleration ranging from 2 to 10 g for a period of 6 minutes (dog Umnitsa, experiment 1, 11 July 1957)

trunk, and for the last 2 minutes it was pressed against the surface of the cradle.

After switching on the centrifuge the pulse rate increased to 155 beats per minute against 130-145 originally (see Figure 4). With the beginning of acceleration a further increase in heart rate was observed, which reached 170-175 in the first 1.5 minutes with a subsequent increase to 190-210-255. A fall and normalization of the heart rate were observed within 5-10 minutes of the end of acceleration.

At the beginning of rotation the frequency of respiration increased by 2-3 times as compared to the original, and attained 37 per minute (against 12-17 originally). Throughout the whole action this was maintained at the level of 24-30. At the end of acceleration the respiration became slower and within 2 minutes returned to the original level.

Experimental results led us to the following conclusions:

1. The effect of variable magnitudes of transverse accelerations (from 20 to 10 g) lasting for 6 minutes, caused in dogs well defined changes in the cardiovascular and respiratory systems.

2. The maximum arterial pressure, as a rule, increased by 50-80 mm Hg and the heart rate exceeded the original value by 1.5-2 times, while the respiration rate increased by 1.5-3 times the original value. The respiration and blood circulation returned to their original values within 5-10 minutes of cessation of acceleration.

3. An increase in respiration and heart rates was noted during the action of the acceleration and was interpreted as preparatory adaptive reactions of the animal body.

4. The above specified durations and magnitudes of acceleration were satisfactorily endured by the animals. Subsequent observations did not show any deviations in their state of health.

Summary

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The paper considers the effect upon animal organisms (dogs) of variable long-termed accelerations acting transversally to the body axis. It has been found that the animals endured accelerations ranging from 2 to 10 g during 6 minutes quite satisfactorily though the accelerations brought about essential changes in main indices of cardio-vascular and respiratory systems. Their functions returned to normal 5 - 10 minutes after the action stopped.

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# THE PROBLEM OF THE BIOLOGICAL ACTION OF COSMIC AND SOLAR RADIATIONS IN THE MOUNTAINS

V. I. Danileyko

It is possible to separate three distinct stages in the history of investigating the biological action of cosmic radiation:

1. The study of the effect of cosmic radiation on plants growing at high altitudes.

2. Research into the effects of cosmic radiation on flora and fauna during stratospheric flights of balloons at heights up to 30 km. Various methods of investigation were used during these flights:

observations of the general condition of humans and animals participating in the flights (Simons, 1958);
genetic studies using seeds of plants, insects and their eggs, etc. (Frizen, 1936; Eugster, 1953, and others);
histological investigations of preserved biological tissues of small animals lifted into the stratosphere in airtight capsules (Eugster, 1953; Campbell, 1954, and others).

3. Radiobiological studies carried out on Soviet artificial satellites. These studies were carried out with the help of numerous methods (physiological, genetic, immunological, psychological, etc.) using biologic material of different levels of evolutionary development: animals; organs; tissues; cells developing outside their intrinsic cellular association, and single cell organisms; organisms with subcellular structure; and, finally, organic material.

The most extensive data which are at present at the disposal of cosmic radiobiology were gathered during experiments on Soviet artificial Earth satellites (AES).

The factual material obtained in non-Russian investigations in stratosphere balloons, in spite of the variety of methods used, is limited and cannot be used as a basis for any conclusion concerning the biological action of cosmic radiation (Slater, 1956). The exceptions are the data presented by J. J. Eugster (1953), and H. Yagoda and H. J. Smith (1954), who detected traces of ionization in biological tissues, as well as the data of H. J. Chase (1955), who observed depigmentation of hair in black mice, which appeared after 2-3 weeks' habitation at an altitude of up to 30 km. In certain cases a loss of pigment (greying) was observed

in individual hairs, and sometimes in groups, occurring in rows, forming small white spots. In the earlier experiments of Chase (1949) it was found that the hair follicles of black mice serve as extremely sensitive indicators of ionizing radiation. On the basis of these data the author came to the conclusion that the changes observed after the flights are caused by the penetration, into the surface layers of the tissue, of heavy nuclei, composing the primary cosmic radiation, and also due to the formation of ionization traces in the integuments.

Although the explanation given here was not supported by histological studies of the adjacent tissues, nevertheless the effectiveness of the depigmentation test when studying the penetrating abilities of heavy nuclei is recognized by many investigators, while experiments with black mice were carried on until quite recently (Baar, 1959).

Under the conditions of flight outside the terrestrial atmosphere, in addition to the action of primary cosmic radiation, the body is affected by a whole series of other factors, namely secondary cosmic radiation, X-rays, and in certain cases also solar ultraviolet rays, change in the concentration and the coefficient of unipolarity of the aeroions in the atmosphere of the cabin, etc. These additional factors are not at at all indifferent for the body, even when they are present at a comparatively low intensity.

As early as 1934, Ye. M. Kreps and N. N. Sirotinin noted in particular that reversible depigmentation (greying) of part of the hair may be observed in high mountain regions, under certain conditions of increased intensity of secondary cosmic and solar radiation and in the complete absence, as was later proved, of heavy nuclei.

It is therefore possible to suppose that the phenomenon of depigmentation in this case may be caused by the action of particles comprising secondary cosmic radiation.

The present work had the following aim:

1. To investigate the possibility of the appearance of depigmentation phenomena in conditions of a small increase in the intensity of radiation.

2. To explain to what extent a small increase in the intensity of cosmic radiation can influence the quantitative and qualitative composition of the cell elements of the blood.

Results

Observations on the depigmentation of hair cover were carried out during an expedition to Mount El'brus in 1957 and 1958. The material received consists of the investigational and in certain cases of the interrogational data of persons participating in the expedition and climbers. It was found that in 10 of them, 2-3 weeks after ascent to an altitude of 2200 m (Terskol) and above, isolated depigmented hairs appeared in the hair cover of their faces and hands; alternatively the whole process of greying was intensified. In 7 persons the process of depigmentation was reversible - after a certain time following descent from the mountain no further grey hairs appeared and the number of the existing grey hairs decreased.

Of the second group which was investigated (15 persons), 6 had no greying hair at all, 9 had greying hair but for various reasons it was not possible to find out whether the numbers had changed. The appearance of grey hair must not be considered a result of old age, since half the persons of the first group were between 21 and 30 years old. These observations were also extended to animals, 1 dog and 22 black mice of the $C_{57}$ strain; during a period of 24 days they lived at altitudes of

2200, 2900, 3500 and 3900 meters above sea level.

The animals received different diets and were protected from the effects of cold.

It was found that in the case of the dog the amount of grey hair on definite parts of its body increased by the end of the expedition up to 23.5, 32.8 and 47.8 percent of the total number of hairs, as compared with 9.2, 21.9 and 10.7 percent in the case of domestic conditions (in each case several hundred hairs were counted on each portion).

In the case of black mice, before the experiment a total of 3 grey hairs only was detected. At the end of the experiment, grey hair appeared in all animals. As an example, a photograph of mouse No. 8 is given, which was taken towards the end of the expedition (Figure 1).

In 2 mice the depigmented areas encompassed groups of hair located side by side, forming greyish spots very closely resembling those described by Chase (1955); one of these areas is shown in Figure 2.

Two of the mice were placed for 10 days at an altitude of 2900-3000 meters screened at the top by a lead sheet 1 cm thick.

Changes in the numbers of the blood elements were studied by the author in 1957 on 9 men (also participants in the expedition) and 30 white mice. It was found in particular that the number of erythrocytes

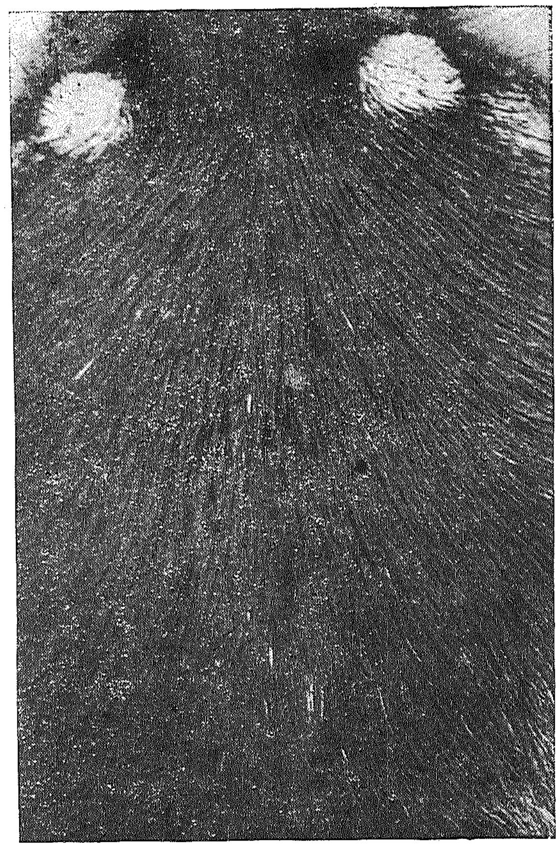

Figure 1. Towards the end of the expedition the fur of animal No. 8 contained 32 grey hairs.

Figure 2. Animal No. 4 was placed for 10 days in a container under a lead sheet 1 cm thick. Towards the end of the expedition, on the back of this animal, two bundles of grey hair were found. One of these is shown in this photograph.

in 1 cubic millimeter of human blood which before the experiment contained 4180 ± 0.04 millions; at a height of 3900 meters, within a fortnight of arrival in the mountains, reached 5212 ± 0.20 millions, that is, increased by 19.8 percent. Then, as a result of acclimatization, in spite of further ascent, the number of erythrocytes started to fall. Changes in the hemoglobin content were at the same time insignificant.

In order to explain to what extent the polycythemia usually observed in the mountains depends on the radiational factor and in particular on the effect of secondary cosmic radiation, two series of experiments were carried out. In the first series the morphological composition of the blood was investigated in 4 white mice which were placed for a period of 10 days at an altitude of 2900-3900 meters inside a container covered with a lead sheet 1 cm thick.

In the second series, the investigations were carried out in identical conditions, but on 6 animals which were placed in a lead container with walls 5 cm thick and a cover of 8 cm (Figure 3).

As is well known, the use of lead plates of a thickness of approximately 1 cm causes a certain increase in the level of ionization in

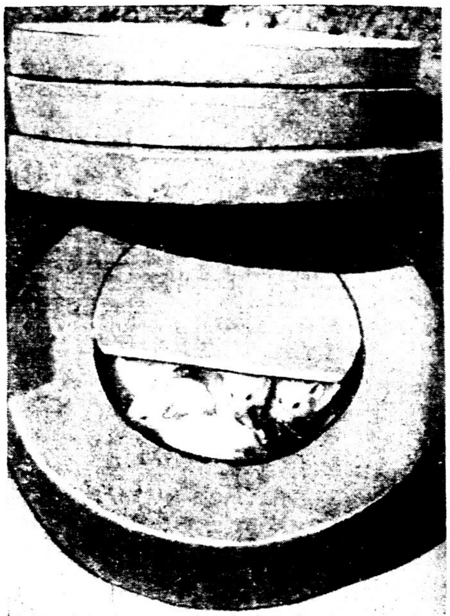

Figure 3. General View of Lead Container with Animals. Cover Removed.

Table 1. Experimental results showing the number of erythrocytes, hemoglobin and leucocytes in the blood of mice exposed to mountain conditions

| Series | Number of Animals | Before the experiment | | | After the experiment | | |
|---|---|---|---|---|---|---|---|
| | | Erythrocytes, millions in 1 $mm^3$ | Hemo-globin, % | Leucocytes, thousands in 1 $mm^3$ | Erythrocytes, millions in 1 $mm^3$ | Hemo-globin, % | Leucocytes, thousands in 1 $mm^3$ |
| First | 4 | $9.33 \pm 0.28$ | $88 \pm 3$ | $8.9 \pm 1.2$ | $9.82 \pm 0.39$ | $98 \pm 5$ | $11.5 \pm 2.3$ |
| Second | 4 | $9.36 \pm 0.27$ | $90 \pm 0.7$ | $12.0 \pm 0.7$ | $9.94 \pm 0.71$ | $103 \pm 6$ | $18.4 \pm 0.7$ |
| Control | 10 | $9.23 \pm 0.14$ | $89 \pm 1$ | $10.0 \pm 0.9$ | $11.48 \pm 0.35$ | $105 \pm 2$ | $16.7 \pm 2.5$ |

connection with the formation of secondary particles, while plates of thickness up to 8 cm eliminate the effect of all particles with energy $10^9$ ev and lower, and absorb approximately 50 percent of the soft component of secondary cosmic radiation (Dobrotin, 1954, and others).

The control animals were placed in a wooden cage. The feeding and the temperature conditions in the nests of all three groups of mice were identical. Mice located in the metal containers were isolated from the lead by thin tin plate and cardboard.

During the various stages of the experiment, some of the animals died during transportation. For this reason the data collected in the table concerning the blood studies refer to the 18 mice which survived to the end of the expedition.

In 1 cubic millimeter of blood taken from the blood vessels of the tail, the number of erythrocytes under domestic conditions comprised on an average 9.33 ± 0.28 millions. Towards the end of the experiment the number of erythrocytes in the animals' blood was 9.82 ± 0.39 millions (first series) and 9.94 ± 0.71 millions (second series), and in the control group 11.48 ± 0.35 millions, that is, on an average higher by 13 percent.

When studying the hemoglobin and leucocyte contents towards the end of the experiment, it was noticed that they had increased in the blood of all three series of animals. The increase in hemoglobin was greatest in the control series and the increase in leucocytes in the second series.

## Discussion

What are the causes of depigmentation of hair cover in high mountain conditions?

These disturbances are not connected either with hypoxia or with increase of the ultraviolet part of the solar spectrum. Most likely they are the results of the action of the components of secondary radiation (electrons, positrons, etc.),acting on the surface tissues.

The marked changes observed in the animals living in the container with the lead cover of 1 cm thickness are highly significant. They were apparently caused by penetration into the surface tissues of the body of particles formed during the interaction of cosmic radiation with the substance of the lid. It may be assumed that the depigmentation was the result of the appearance within the focus of ionization of the atoms of hydrogen, hydroxyl radicals, hydrogen peroxide, and possibly other substances.

However, if the greying of hair is caused by a cosmic shower resulting from the so-called transitional effect in the absolute absence of heavy nuclei, then it is completely clear that this test may be used during high-altitude and cosmic flights solely to observe the overall effect of the particles comprising the cosmic radiation on the body.

Among the causes producing polycythemia in mountain conditions, the increase in the intensity of solar radiation, the ionization of the air, etc., all play a certain part. At one time it was even considered that the change in the cell content of blood at a high altitude depends mainly on the increased effect of solar rays. The influence of the sun on erythropoiesis was confirmed by many investigators (Sirotinin, 1927; Yegorov, 1928, and others). The mechanism of its action on the haemopoietic organs of living creatures has not so far been completely explained. It is customary to think that this action is mediated and that the nervous and endocrine systems constitute main intermediary links.

However, during "ascents" in pressure chambers, in mountains and in aircraft, it was found that the main factor determining the reaction of the haematopoietic system in these conditions is the lowering of the partial oxygen pressure in the air. The investigational results of the cell elements of blood are often variable, since most of the investigations only take into consideration oxygen pressure and do not consider the effect of radiational factors, which increase rapidly with altitude. Furthermore, the extent of the influence of different radiations under conditions of ascent in the mountains is not as yet fully clear, although the need for such studies has been stressed by numerous authors (Zhukov, Frank, 1936; Van Leer, 1947).

Summarizing the data obtained as the result of observations of the haematopoietic functions, we came to the conclusion that the considerable difference in the erythrocyte content of the blood of the experimental and control groups of mice was caused by the effects of solar radiation. No consequences of screening some of the animals from soft components of cosmic radiation could be detected; this may be explained either by insufficient observation or by the absence of fine techniques of investigation, as well as the extremely insignificant level of the cosmic radiation. The ionization of air at an altitude of 3-4 km caused by the radiation is equal to 0.3-0.5 mrep per day, which exceeds only by 3.5 times the ionization at sea level.

Summary

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1. Observations made upon people and animals that remained in mountains for long periods at an altitude of 2200-3900 m above sea level demonstrated that in such cases depigmented (gray) hairs appeared or their number increased.

The phenomenon of hair depigmentation in people and animals is evidently a result of the action of cosmic radiation particles on surface tissues of an organism. The high sensitivity of hair follicles of black mice to ionizing radiations makes it possible to employ the animals as biological indicators of cosmic radiation.

In the case of the appearance of gray spots on the hairy coat of the animals the conclusion that heavy nuclei of cosmic radiation have struck the tissue can be drawn only if ionization traces are found there histologically.

2. The mice that stayed in an open cage at the altitude of 2900-3900 m for 10 days showed a 13% increase in erithrocytes as compared to the animals protected from solar radiation. This confirms the idea previously suggested by various authors, of the influence of solar radiation upon the hemopoietic function of bone marrow both in usual and mountain conditions.

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# METHODS OF INVESTIGATING THE BLOOD CIRCULATION OF THE BRAIN UNDER CONDITIONS OF A MODIFIED GRAVITATIONAL FIELD

Yu. Ye. Moskalenko, R. M. Bayevskiy and O. G. Gazenko

One of the results of the influence of a modified gravitational field on human and animal organisms is the redistribution of blood. Under these conditions the blood circulation of the brain can be perturbed and, consequently, also the normal functioning of the central nervous system. Experimental investigation of the blood circulation in the brain under conditions of a modified gravitational field is therefore of great practical importance for space flights. This work describes methods of recording intracranial electroplethysmograms of animals under conditions of prolonged experiment and during experiments on a centrifuge.

An electroplethysmogram is a waveform representing changes in the electrical resistivity of tissues caused by displacement of blood. Since the electrical conductivity of tissues is several times lower than that of blood, the inflow of blood is accompanied by a reduction in resistance and its outflow by an increase. The intracranial resistance depends also on the movement of plasma (Moskalenko, Naumenko, 1957). For recording intracranial electroplethysmograms in animals, grafted-in electrodes are employed. These electrodes are in the form of perspex probes with a silver plate and are screwed into trepanned apertures until they make contact with the dura mater. Shielded by polythene tubing, the leads from the electrodes are led under the skin and taken out at the back or neck of the animal.

For registering intracranial electroplethysmograms, a transistorized portable electroplethysmograph was used. The diagram of the instrument is shown in Figure 1. The device consists of: a driver-generator with a buffer amplifier, a bridge, a carrier frequency amplifier and a detector. The first unit operates as an inductively coupled oscillator producing sinusoidal oscillations of 30 kc/s. The buffer amplifier protects the oscillator from the load and matches it to the internal resistance of the bridge. The bridge is balanced capacitively and resistively. The carrier frequency is amplified by a two-stage wideband amplifier having an overall gain of about 1000; the gain of the amplifier changes, depending on the input voltage. In this way, a logarithmic scale is achieved for high input voltages which enables investigation at a constant gain of the signals corresponding to small fluctuations in the blood inflow (pulse and respiration waveforms in the vicinity of the balance point of the bridge) as well as large changes in the blood inflow. The output unit of the instrument is in the form of a bridge-type rectifier. The output

voltage for the device varies from 0 to 8 V; the instrument is supplied from a DC source of 24-27 V and uses 5-8 mA; its weight is 300 g.

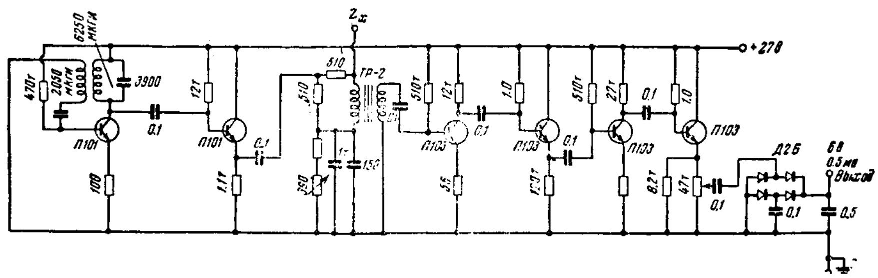

Figure 1. Basic Electrical Circuit of the Portable Electroplethysmograph

After laboratory development of surgical technique and investigations on several dogs with permanently grafted-in electrodes, centrifuge experiments were commenced. Figure 2 shows the intracranial electroplethysomogram of the dog, Chernonos, taken on the eighth day after the operation. The curve clearly shows pulse waveforms and small oscillations caused by the movement of the dog. During experiments on the centrifuge, the amplification of the instrument was reduced in order that the large variations in the intracranial resistances caused by the blood flow would not exceed the scale limits of the instrument (Figure 3). The most interesting data were obtained during overloads in the direction of "head-pelvis" of the animal, achieved by suitable positioning of the animals on the centrifuge chair. The polarity of the recording was chosen so that an increase in their resistance resulted in an upward movement of the recording pen and a reduction in resistance in a downward movement. Figure 3 shows the results of one of the experiments: the effect of the overload of up to 1.5 g along the axis "head-legs". As is seen from the recording from the beginning of the rotation to the instant of reaching an overload of 1 g, individual increases in the intracranial resistances were observed which corresponded to a reduction in the blood filling of the brain tissues. The changes in resistance were temporary

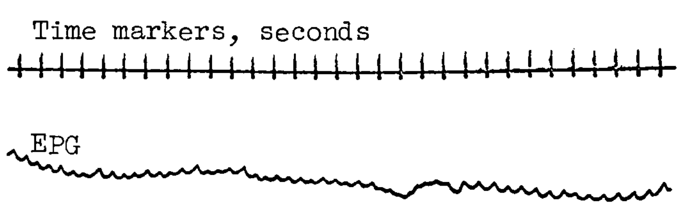


Figure 2. Intracranial Electroplethysmogram of the Dog, Chernonos, Taken on the Eighth Day after the Operation

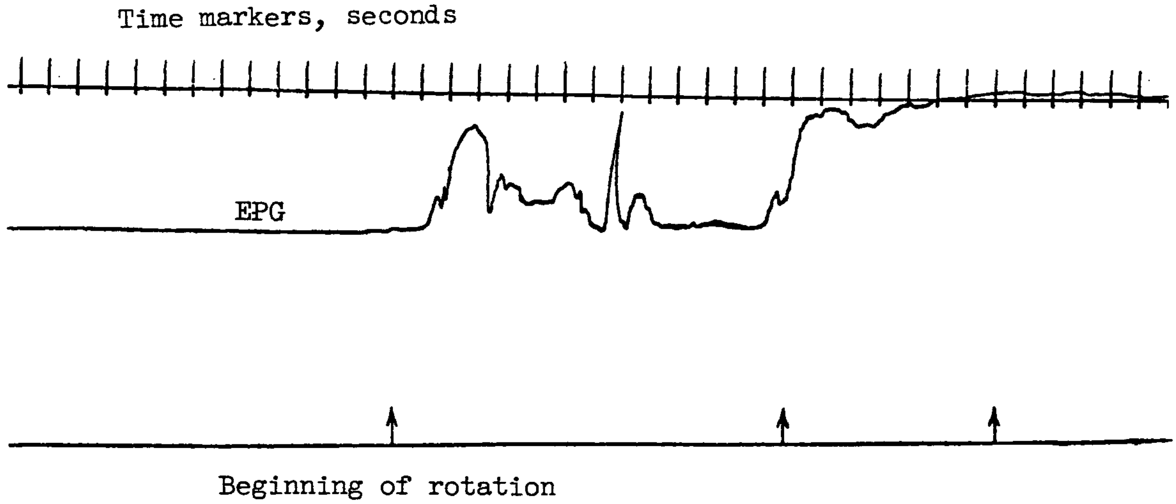


Figure 3. Changes in the Electroplethysmogram of the Dog, Chernonos, During Overloads up to 1.6 g (Along the Axis "Head-pelvis")

and it is possible that they reflected a process of compensatory adjustment of the brain blood circulation to the new conditions. With overloads increasing above 1 g a clear increase in intracranial resistance was observed which obviously indicated commencement of blood redistribution caused directly by the action of the centrifugal forces. These experiments confirmed the applicability of the method of intracranial electroplethysmography to the investigation of the blood circulation in the brain under conditions of a modified gravitational field and enabled making recommendations regarding the design of electroplethysmographic equipment aboard the capsule and the feasibility of conducting similar experiments under conditions of space flight.

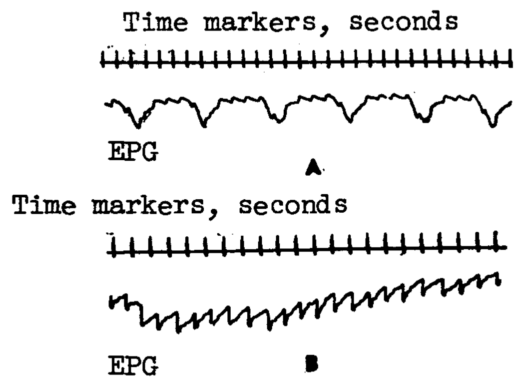


Figure 4. Electroplethysmograms of Various Portions of the Human Body

A - Thorax; B - Forearm

Simultaneously, experiments were carried out on the use of a portable electroplethysmograph for investigating the blood filling-in various portions of the human body. Figures 4a and b show the characteristic electroplethysmograms of the thorax and forearm. The first of these shows marked respiratory waveforms. The electroplethysmogram of the head obtained with electrodes situated at the temples is of some interest (Figure 5). It is seen that the resistances decrease on lowering of the head, which corresponds to an inflow of blood to the brain. Lifting of the head and outflow of blood from the brain cause an increase in the resistance of the tissues. Since the changes in the blood filling of the main brain cannot be investigated by means of the usual plethysmographic methods, electroplethysmography is unfortunately the only practical method of studying the intracranial blood circulation in humans.

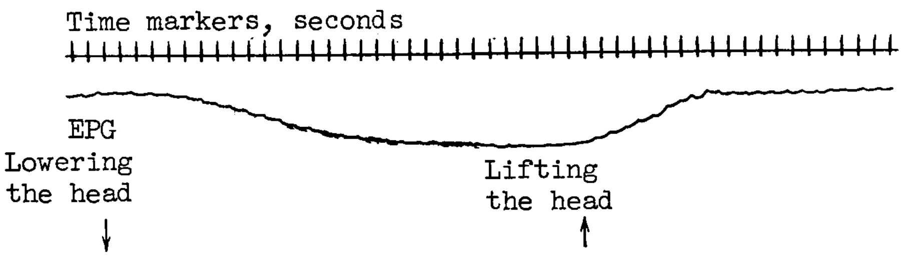


Figure 5. Changes of the Intracranial Electroplethysmogram of a Man During Lowering and Lifting of the Head

Use of intracranial electroplethysmography in field experiments on animals was discussed in detail by A. A. Kedrov and A. I. Naumenko (1954). The experimental work of the present authors with these methods fully confirm the possibility and desirability of their use, not only for investigating the intracranial blood circulation of animals under conditions of prolonged experiments, but also as a method of investigation and control during manned flight in space.

Summary

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A description is given of a transistorized electroplethysmograph and its use for recording intracranial electroplethysmograms in dogs undergoing prolonged experiments and centrifuge runs.

Some information is given on applying the electroplethysmograph for investigating the degree of blood filling various parts of a man's body. Recommendations are made on using the described method during space flights.

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# CONTAINERS FOR CERTAIN BIOLOGICAL MATERIALS USED IN EXPERIMENTS IN SPACESHIPS

N. V. Baranovskaya and A. A. Gyurdzhian

To study the genetic effects of cosmic radiation and other flight factors, various organisms were used in experiments on the second and subsequent satellites: bacteria, green plants (spiderwort), grain, water plants, insects (Drosophila), mice, etc. (Stantsii v kosmose (Space Stations), 1960).

To conduct this type of investigation on spacecraft satellites, special experimental procedures are required and containers have to be used which will insure conditions of viability and the possibility of observing the state of the biological objects.

A convenient material for such containers is transparent Plexiglas. Plexiglas containers produced for this purpose satisfy the following requirements of prolonged flight experiments (which are accompanied by powerful flight factors, such as acceleration, vibration, etc.):

a. access of light and air to the biological material;
b. conserving the viability of the material;
c. observation of the material (e.g., during blooming of the spiderwort) which are inside the containers (before, after or during the flight by means of television and a cinefilm);
d. stability of the containers (the walls should not be brittle) and prevention of spilling the biological material and the contents of the containers (nutritive media, liquid, etc.) into the capsule, onto the instruments, etc.;
e. as low as possible attenuation of the extraterrestrial ionizing radiation by the walls of the containers (see illustration).

1. The container for the spiderwort is cylindrical in shape and consists of two beakers 150-170 mm high, 70-75 mm diameter with a wall thickness of 1-2 mm. The top beaker should slide freely into the bottom one with very little clearance between the walls.

A layer of garden soil 70-80 mm high is placed into the bottom beaker and the plants (spiderwort) are bedded into the soil. The entire surface of the soil surrounding the plants is covered first with gauze, then with a metallic net, and again with a layer of gauze. Next, without damaging the flowers and foliage, the plants are covered with the top beaker, the edges of which press onto the net covering the soil. The

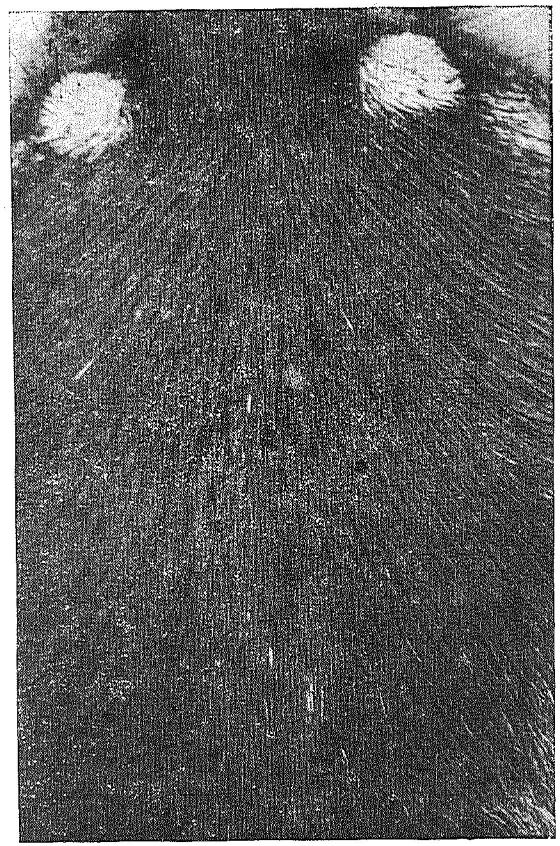

Figure 1. Towards the end of the expedition the fur of animal No. 8 contained 32 grey hairs.

Figure 2. Animal No. 4 was placed for 10 days in a container under a lead sheet 1 cm thick. Towards the end of the expedition, on the back of this animal, two bundles of grey hair were found. One of these is shown in this photograph.

The described containers were used on the spaceships and satellites. No damage or disturbance could be detected in the biological material or the containers on return to Earth.

Summary

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Plexiglas containers used for carrying various biological material during the spaceship experiments, i.e., green plants, Drosophila, spiderwort, seeds and seedlings, Actinomycetes and Chlorella are briefly described. The containers met all the requirements of prolonged flight experiments. They allowed free access of light and air, and their transparency made it possible to observe the material by means of cine or television cameras. The above biological material and containers suffered no damage and were used successfully in the second and subsequent Soviet satellites.

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# ENSURING LIVING CONDITIONS FOR MICE DURING PROLONGED FLIGHT IN SPACESHIPS AND SATELLITES

I. V. Baranovskaya and A. A. Gyurdzhian

Study of the biological effects of various factors encountered during space flight (cosmic radiation and other forms of ionizing radiations, accelerations, vibrations, prolonged states of weightlessness) presents a very extensive general biological problem, the solution of which requires the use of various representatives of the animal world. In this respect, laboratory mice are especially convenient biological objects.

Their small size and relatively simple environmental provisions make it possible to carry out the flight experiment on mammals where there is limited space and weight. Their fast reproduction also makes it possible to obtain genetic data for statistical analysis.

Provision of the necessary environmental conditions for the mice during an extended flight (lasting a number of days) in the pressurized capsule of a spaceship in conditions of weightlessness and acceleration necessitated the design of special containers and experimental techniques. In particular, it was necessary to envisage provision of suitable food and water during the whole experiment (Kratochvill et al., 1959; Space Stations, 1960). This flight experiment imposed a number of requirements with regard to the resistance of the equipment to certain flight factors, such as acceleration and vibration.

The container for the mice was made of metallic netting on a wire frame, the size of the mesh being approximately 5 x 5 mm. The container is in the form of a parallelepiped cage 170 x 120 x 120 mm, the front wall being detachable and braced by means of two bolts (see figure).

Down both sides of the container, along all its lengths are placed three, and at the top two, cylindrical feeding troughs. These troughs are made of 16 mm diameter duralumin tubes. The side facing the container carries 10 mm wide openings which do not quite extend to its end. The netting in the wall of the container facing the troughs also had corresponding cutouts (these were made by removing a few of the horizontal wires). After the trough is charged with food, it is shut by means of a lid and inserted in the corresponding holes of two brackets which gird the container. The troughs are securely fixed to prevent their rotation around the longitudinal axis during flight. A wire is threaded through the openings in the frontal part of the tube and the corresponding openings in the lid in order to secure the latter.

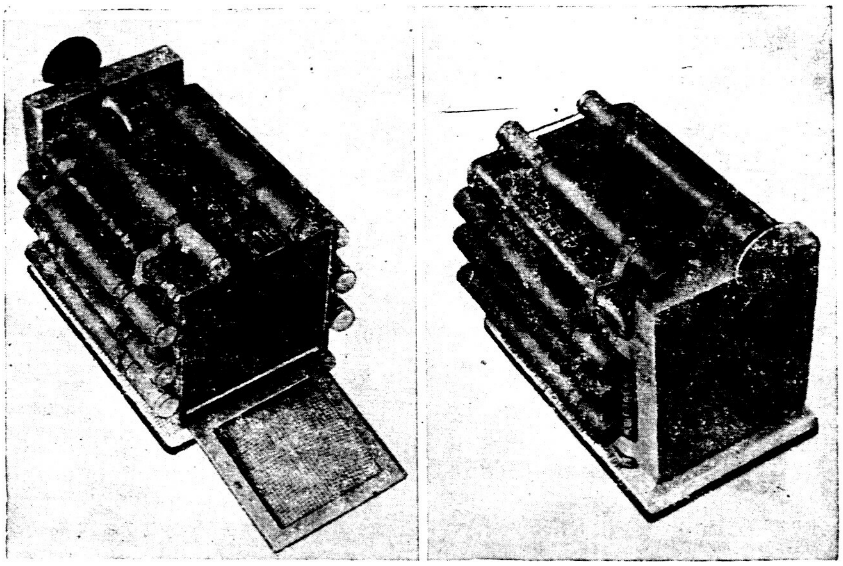

Figure. Containers for Mice

Open and Shut View

A rectangular tank for water is attached to the rear wall of the container. This tank has a capacity of 250-300 ml. At the rear of the upper part of the tank there is a small trapdoor for filling it with water (diameter approximately 20 mm) which can be hermetically shut by means of a lid with bolts and a rubber washer. An S-shape tube is welded to the top of the frontal wall of the tank and extends inside the container for the mice. The internal diameter of this tube is approximately 5 mm. A wick is threaded through the tube, one end of it (approximately 1 m long) filling the whole volume of the tank. The width of the wick is so chosen that the water issues at the rate of 1 to 2 drops a minute. For that purpose a bandage of an average width of 10 cm was usually found to be useful. It was cut off close to the outer end of the tube.

The feeding troughs were filled with special food briquetted in the shape of small cylinders of the appropriate diameter. The briquetted food concentrates for mice were developed by the Academy of Med. Sciences USSR. They contained all the necessary nutritious ingredients and could be stored for a long period of time. The tank was filled with water shortly before the experiment. For preserving the water 0.05 g per liter humazine was added. Ten to fifteen mice were placed in the container and and the front wall was replaced. A few pieces of fresh carrot and beetroot were also placed in the cages during the experiments on the spaceship-satellites.

The container was mounted on a strong textolite plate by means of 4 legs (using bolts with bushings). At a distance of 1 cm from the lower surface of this plate a moisture absorbing plate was placed which was covered with a layer of netting to prevent the mice reaching the swollen-up moisture absorbing substance. A net was fixed on top of the trough with the food and on the net a layer of moisture absorbing substance (lignine, filter paper, etc.) was placed. The whole container with the mice was wrapped in one layer of muslin (gauze) to prevent drops of moisture and crumbs falling into the pressurized capsule and on to instruments, etc. The designed container for mice offers a number of experimental and methodological advantages during tests on artificial satellites and spaceships.

The net wall makes it possible for the mice to move freely throughout the whole cage during the state of weightlessness and to consume the briquetted food. The free exchange of air between the container and the pressurized capsule of the spaceship provides the mice with the necessary atmosphere and temperature. The net wall makes it possible to observe the mice under laboratory experiments before the launching and after the return. This method does not prevent the use of cinecamera or television during the flight.

The water provision is fully functional under all conditions of flight, since the water passes through the wick not by gravitation but by capillary action which is independent of the state of weightlessness. Mice learned very quickly how to suck the water from the wick. In the preliminary laboratory experiments, mice endured satisfactorily a residence of 10-15 days in the containers with the appropriate food and water supply.

The above described container and technique for providing living conditions for mice were used during the flights of the second and subsequent Soviet satellites and spaceships. No injuries or changes in the behavior or weight of mice were observed on completion of the flight. The food briquettes were partly eaten during the flight. As is known, the flight of the second spaceship lasted for only 25 hours. Further studies of the above methods in more prolonged space flights are required.

Summary

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A detailed description of containers for mice used during satellite flights is given, together with a feeding device for dispensing briquetted food.

Each container carried 10-15 mice and was 170 x 120 x 120 mm in size. The food dispenser comprised a number of tubes placed along the wire

network of the walls. Water was supplied separately through a wick mechanism. A particular feature of the latter was that the rate of water supply was purely a function of the capillary effect and was independent of any such factors as accelerations, jerks, weightlessness, etc. Free air ventilation between the container and the pressurized capsule, as well as humidity control, were also provided.

The above system proved successful in every respect and was used during the 25-hour flight of the second satellite. Author

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11704



# METHOD OF "INTEGRATED" PHONOCARDIOGRAPHY

R. M. Bayevskiy

The method of phonocardiography has been widely used in clinical practice during recent years. The recording of sound effects which accompany cardiac activity makes it possible to investigate the important function of hemodynamic parameters, the duration of the mechanical systole, synchronism of contraction of the left and right chambers of the heart and operation of the valves. In space medicine this method of investigation is undoubtedly of interest. The recording of phonocardiograms under conditions of prolonged weightlessness is particularly important.

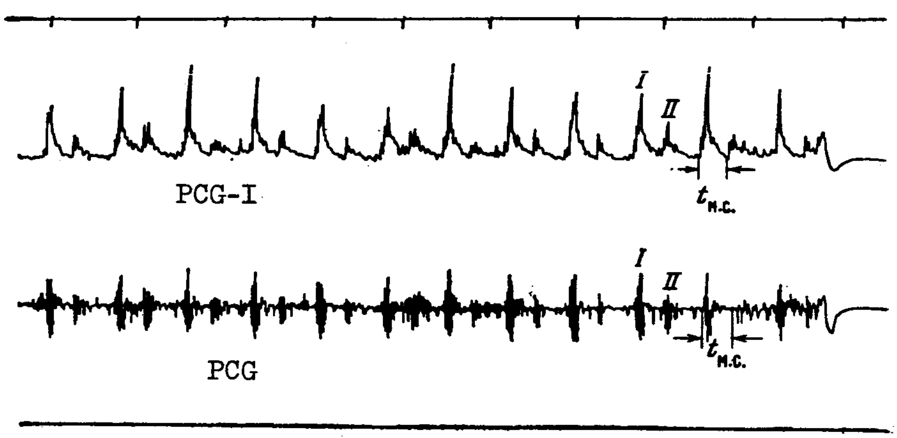


Figure 1. Synchronous Recording of the Usual (PCG) and "Integrated" (PCG-I) Phonocardiograms

I - First Tone; II - Second Tone; $t_{M.C.}$ - Duration of the Mechanical Systole

However, use of phonocardiography in space flights is very difficult due to the necessity of transmitting signals by radiotelemetering channels. The difficulty lies in the fact that for transmitting a phonocardiogram the necessary channel capacity is three times higher than that required for transmitting electrocardiograms.

In this work methods of "integrated" phonocardiography are described which rely on separation of the low-frequency envelope of audiosignals by means of detection and integration of output signals of the phonocardiographic amplifier. In this operation the information relating to the

frequency content of the phonocardiographic waveform is lost but it is still possible to determine all the parameters related to the functioning of the heart muscle (duration of the mechanical systole, mechanico-electrical factor, and so on). Radiotelemetering channels of considerably smaller capacity than that required for transmitting the usual phonocardiogram can be used for an "integrated" waveform.

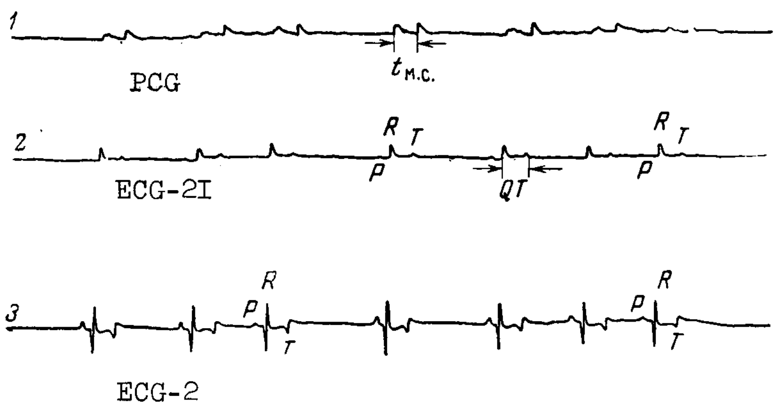


Figure 2. Synchronous Recording of the "Integrated" Phonocardiogram (1) and Electrocardiogram in Standard II

(2 - Usual Recording; 3 - "Integrated" Recording)
I - First Tone; II - Second Tone; $t_{M.C.}$ - Duration of the Mechanical Systole

It is not necessary to employ special microphones for recording "integrated" phonocardiograms. The author uses a miniature telephone-type TG-7 (50 ohm resistance) without special modifications and an amplifier having a gain of about 20,000 and a bandwidth of 50-500 cps. The output of the amplifier was followed by a detector-type D7Zh and an integrating network with a time constant of the order of 0.02-0.05 sec (resistance of 2 kohm and capacity of 20 $\mu$F). Figure 1 shows the usual and the "integrated" phonocardiograms, which were recorded synchronously. The methods of "integrated" phonocardiography were used successfully in experiments with dogs. Phonocardiograms were recorded simultaneously with electrocardiograms (Figure 2). The sensor-transducer was fixed in the region between the fourth and fifth ribs at 2-4 cm to the outside from the left edge of the breastbone or above the apex of the lungs. The transducer was fixed onto the animal by bandage.

Apart from undistorted transmission by narrow-band radiotelemetering channels, the following characteristic of "integrated" phonocardiography can be of interest to physiologists and physicians:

1. Recording of integrated phonocardiograms can be effected by recording instruments with a low natural frequency, including very crude pen-recorders.

2. Respiration and small displacements do not affect the quality of the recorded waveforms.

3. Frequency filters are not necessary and telephone earpieces can be used as a microphone.

Integrated phonocardiography is a simple and reliable method of investigating the functioning of the heart system and can be used not only for space medicine but in physiological laboratories and clinics.

Summary

11704

The technique of "integrated" phonocardiography was developed for the purpose of lowering the capacity of the telemetry channel required for the transmission of phonocardiograms. It relies on separation of the low-frequency envelope of audiosignals by detection and integration of output signals of the phonocardiograph amplifier. As a result, the information relating to the frequency content is lost but it is still possible to determine all the parameters related to the functioning of the heart muscle. Consequently, telemeter channels with a capacity considerably lower than that required for transmitting the usual types of phonocardiograms can be used.

Hetherin

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11705



# A TECHNIQUE FOR RECORDING SPHYGMOGRAMS OF DOGS

R. M. Bayevskiy and V. I. Polyakov

Sphygmography is one of the oldest methods of investigating the activity of the heart. This method, together with oscillography of the arteries, yield information on the state of the peripheral blood circulation and thus its use under conditions of spaceflight is recommended for studying hemodynamic shifts during overloads and weightlessness.

A simple technique for recording sphygmograms of the carotid and caudal arteries of dogs is described; this is based on the use of "tenzolit" pickups - rubber tubes containing carbon powder. Such sensors are parametric and require an external power supply. The circuit diagram of the transducer is shown in Figure 1. The internal resistance of the transducer is between 200 and 700 ohm and the overall current of the measuring circuit does not exceed 1-2 mA. The buffer capacitor $C_1$ together with the resistance $R_2$ and the capacitance $C_2$ form a simple passband filter which limits the level of low and high frequency noise. The useful signal at the output of the amplifier can reach several mV.

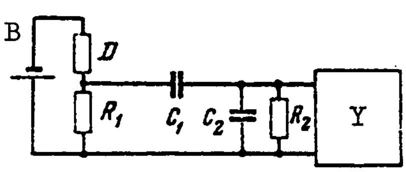


Figure 1. Circuit Diagram of the Transducer Connection

B - 1.5 V Battery; $R_1$ - 500 to 1000 ohm resistance; $R_2$ - 100 kohm; $C_1$ - Capacitance of 0.1 to 0.5 $\mu$F; $C_2$ - 0.2 to 1.0 $\mu$F; Y - Amplifier; D - Sensor

The transducer is in the form of a sleeve which is inserted into the carotid artery and is taken out into a skin flap. The "tenzolit" element mounted in the sleeve is closely pressed to the vessel and thus records all the movements of its walls. Figure 2 shows a sphygmogram of the carotid artery recorded simultaneously with a cardiogram. Similar recordings were obtained by means of transducers in which the "tenzolit" element

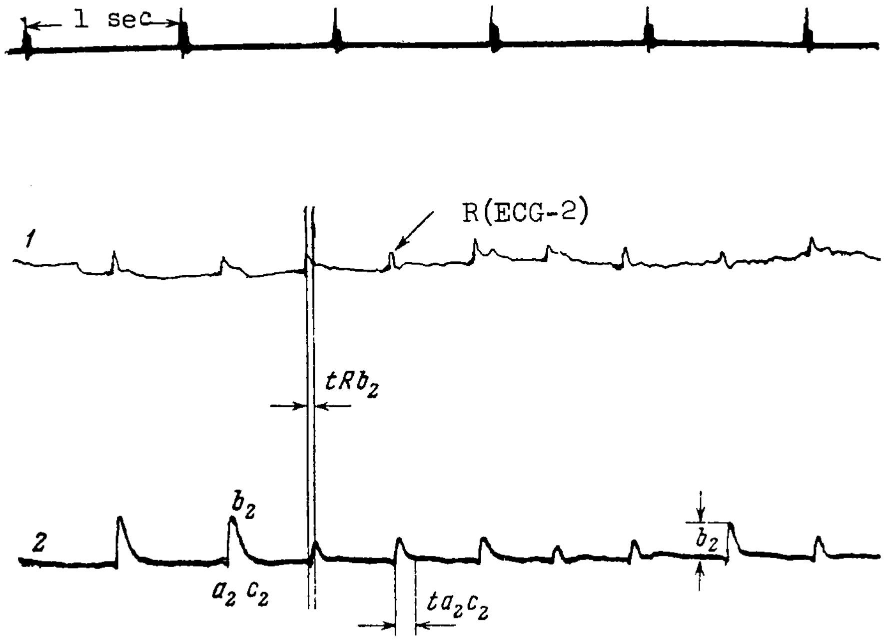


Figure 2. Sphygmogram of the Carotid Artery

1 - Electrocardiogram of the Second Experiments;
2 - Sphygmogram of the Carotid Artery;
$a_2$, $b_2$, $c_2$ - Points on the Comparison Time Scale of the Sphygmogram;

$tR\ b_2$ - Relative Rise Time of the Pulse Waveform;

$ta_2c_2$ - Duration of Pulse Cycle

was replaced by a piezoelectric crystal. The sleeve-type sphygmographic transducer gives stable recordings since it is little influenced by movement of the animal or even by external vibrations.

To record the pulse of the caudal artery a transducer in the form of a ring, situated at the base of the tail, was designed. In this method, however, the quality of the recording was affected by the movements of the animal. Pulse waveforms of the front and hind extremities of the dogs were also obtained by means of a ring transducer. A ring "tenzolit" transducer can be used for recording the pulse in humans from a finger, forearm or lower leg, etc. (Figure 3). Such recordings are not really sphygmograms since changes in the resistance of the ring transducer are caused by changes in the size of the circumference, i.e., by volume variations of the blood-filled regions of tissues where the transducer is situated. Consequently, this is a plethysmographic transducer but the electrical differentiation (see the circuit) brings out the pulse

component of the plethysmogram only. Respiratory variations in the recorded pulse waveforms can serve as a source of additional information regarding the frequency and depth of respiration.

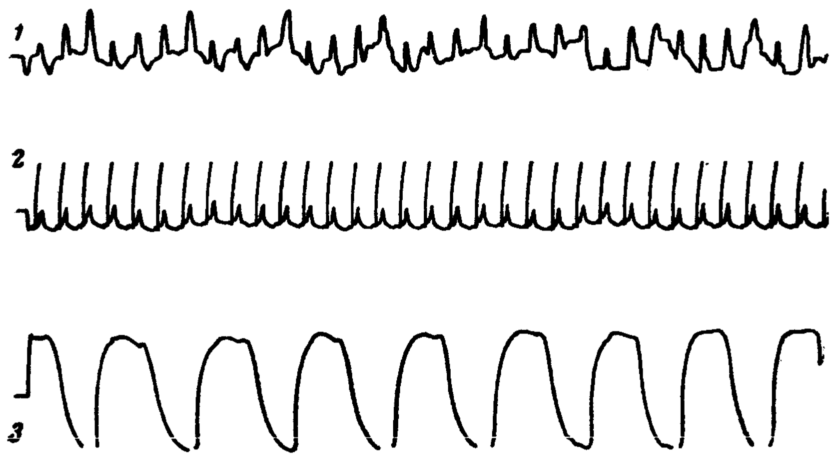


Figure 3. Simultaneous Recording of the Pulse from a Human Finger by Means of a Ring Transducer (1), an Electrocardiogram (2) and a Pneumogram (3)

Summary

11765

A technique is described for measuring changes in the blood pressure of dogs during space flight. Measurement is made by means of rubber tubes or sleeves containing carbon which record changes in the movement of the walls of the carotid artery. The movement can be converted to an electrical signal which is amplified and traced. Blood pressure changes in the caudal artery are measured by a similar device in the form of a ring.

Author

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# RECORDING OF SEISMOCARDIOGRAMS IN DOGS

R. M. Bayevskiy and L. A. Kazar'yan

Study of the mechanical manifestations of cardiac activity is of great importance in hemodynamic investigations. A wide use of ballistocardiography has been witnessed in recent years; this method is based on recording the body movements which occur as a result of cardiac contractions and transfer of a mass of blood into the large vessels. A ballistocardiogram gives information on the strength and coordination of cardiac contractions, i.e., on the state of contractional function of myocardium. The study of these factors under spaceflight conditions is of considerable interest, especially as regards the effect of weightlessness on hemodynamics. However, the use of ballistocardiography in the investigations on dogs is extremely difficult due to the necessity of anesthesia (Maryev, 1958) or special training (Fedoseyev, 1958).

The ballistocardiograms of dogs under the conditions of free movement with minimum restraint, were obtained by using a seismic method for recording the cardiovascular forces. In this case the sensing element is a seismic transducer which comprises an inertial mass elastically coupled to the object to be measured, and a system converting the movements of the mass into electrical signals. The advantages of seismic transducers lie in their stability, reproducibility of results and absence of any special adjustments.

The constructional details of a seismocardiographic transducer on dogs are given (see Figure 1). The transducer is in the form of a metal box of 60 x 50 x 20 mm, with a base plate made of pertinax ("getinaks") or perspex to which is fixed a flat spring carrying the seismic mass, and this serves simultaneously as the magnetic element of the transducer system. The second element of this system consists of two inductance coils with iron cores, which are attached to the base of the transducer and are fixed relative to the moving seismic mass.

The transducer is placed on the animal's back. The movements of the body caused by cardiac contractions result in displacements of the transducer enclosure relative to the seismic mass. The inertial forces deflect the spring and the seismic mass executes natural damped oscillations. The natural frequency of the oscillations is 22 cps and the damping constant is approximately 0.1 sec. In this manner, each pulse-actuated movement of the body is accompanied by a natural oscillation cycle of the seismic mass and the appearance of corresponding electrical signals at the output of the transducer. The amplitude and duration of each oscillation train (other conditions being equal) depend on the

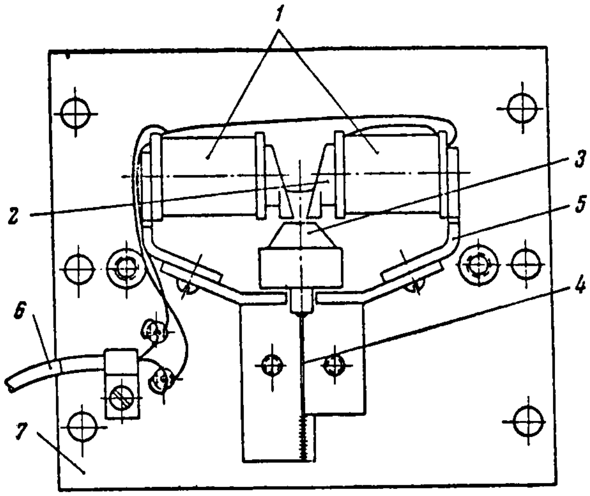


Figure 1. Construction of Seismocardiographic Transducer

1 - Inductance coils; 2 - Core; 3 - Seismic mass-magnet;
4 - Spring; 5 - Magnetic circuit; 6 - Transducer output;
7 - Transducer enclosure

magnitude of acceleration imparted to the transducer and on its operating time.

The amplitude of the seismographic curve is directly proportional to the strength of cardiac contractions. The stronger the contraction, the higher the mechanical energy generated by the cardiovascular system and the higher the acceleration imparted to the transducer. As can be seen from Figure 2, a seismocardiographic waveform consists of two clearly


Figure 2. Electrocardiogram (1) and Seismocardiogram (2) of the Dog Al'fa

defined parts: systolic and diastolic. The time interval between the beginning of each of these is equal to the duration of the hemodynamic systole. Variations of the normal time pattern between the cardiovascular forces results in a lengthening of the damping constant of the natural oscillations of the seismic mass or in the appearance of new oscillation trains. This is observed as an increase in the duration of the diastolic or systolic portions of the waveform and leads to the merging into a single train or leads to the appearance of additional oscillations. All these changes in seismocardiographic waveforms can be regarded as signs of disturbance in the coordination of cardiac contractions.

Seismocardiography is a form of ballistocardiography. The frequency of natural oscillations of the seismic mass is chosen so that it partially coincides with the frequency of the ballistic forces. Due to this, the respiratory movements and other slow displacements of the body do not appreciably affect the measurements. In view of the fact that the spring system of the transducer has only one degree of freedom, only the movements in one direction are recorded (along the longitudinal axis of the animal). Good quality records as a rule are obtained when the animal is completely relaxed.

The recording of the seismocardiograms is impossible during movements but the rapid decay of the natural oscillations of the seismic mass permits recording individual ballistic cycles or a sequence of cycles during periods of rest between the movements. Decoding of the records is considerably facilitated by a simultaneous recording of an electrocardiogram.

Summary

11706

The fundamentals of seismocardiography are briefly discussed and the constructional details of a sensor working on the principle of an electromagnetic-inertial transducer are given.

The above transducer was designed for a dog and was built in a small (60 x 50 x 20 mm) metallic box containing a flat spring carrying a seismic mass serving also as magnetic element of the transducing system. Two induction coils serve as the secondary elements of the system. The transducer was placed on the spine of the animal and the working parameters were so chosen that the cardiac contractions could be recorded without any motor disturbances. Well defined systolic and diastolic portions of the seismocardiogram are shown on the example of the dog Al'fa. The above tracing is compared with the ordinary ECG tracing.

It is concluded that a seismogram permits measurement of the strength and coordination of cardiac contractions, although it is preferred to carry it out parallel with the electrocardiogram tracings.

Author

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# THE CHOICE OF LEADS AND THE ANALYSIS OF ELECTROCARDIOGRAMS IN DOGS

R. M. Bayevskiy and M. M. Osipova

The use of dogs as experimental animals in medical and biological investigations in rockets and artificial earth satellites presents problems to the physiologist which are connected with the standardization of reception methods and in particular of those methods which are used in electrocardiography. As is well known, in questions of the choice of leads and the analysis of electrocardiograms in dogs, there has been until now no clear-cut and uniform opinion.

In order to standardize electrocardiographic procedure we have performed approximately 500 recordings on 20 dogs. The recording was carried out using type 4 PFD-7 apparatus by means of 7 needle electrodes which were placed in 3 limbs and in 4 chest positions, G-1, G-3, G-5 and G-9. In each of the dogs under investigation 18 electrocardiographic leads were recorded, namely, 1, 2, 3, UN, UP, GP-1, GP-3, GP-5, GP-9, GN-1, GN-3, GN-5, GN-9, GL-1, GL-3, GL-5, GL-9. The experiments were all carried out at the same time of day. All the animals were examined lying in the prone position.

As is well known, electrocardiograms in dogs are characterized by marked respiratory arrhythmia and considerable variation in the form and amplitude of the waves, which are not due to the action of any factors upon the animal. To simplify the interpretation of the above features, we introduced the arrhythmia index ($\Delta$RR) and the structural formulas of the complexes. The arrhythmia index was determined by subtracting the minimum duration of the cardiac cycle from its maximum duration ($\Delta RR = RR_{max} - RR_{min}$). The structure of the electrocardiographic complex was defined by letters according to the number of waves present. Small letters indicated a reduction in the waves, capital letters indicated normal magnitude, and capital letters in brackets indicated an increase in the waves. In splitting, sharpening or biphase peaks, the corresponding letters had conventional signs written above them (V), ($\Lambda$), (+ -). Displacements of the PQ or ST intervals were designated by horizontal lines above or below the letters. Figure 1 represents samples of tracings obtained with the dog Shutka. Under the complexes are shown their structural formulas. All varieties of complexes may be reduced to five typical structures (Figure 2).

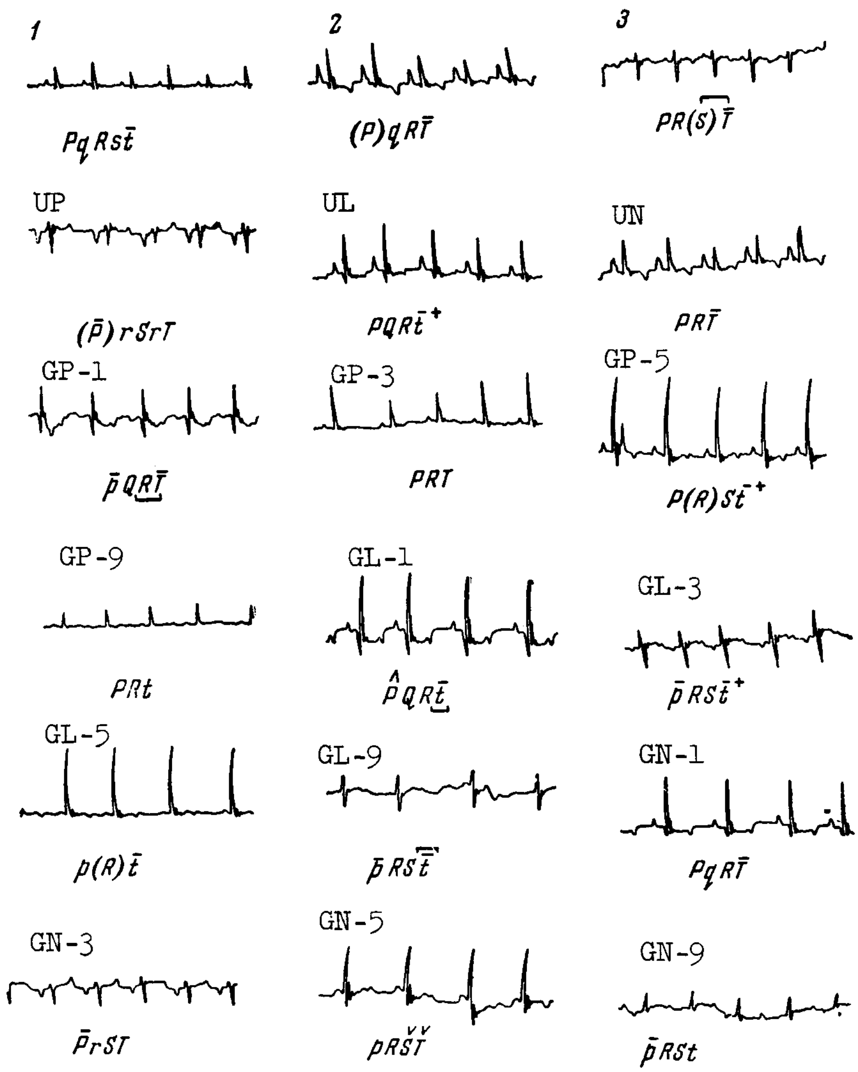


Figure 1. Electrocardiogram of the Dog Shutka in 18 Leads

In contrast to the ECG in man, splitting, double phase or inversion of the waves in dogs cannot serve as pathological indicators. We are of the opinion that only the changes in the structural type of the complex as a result of one influence or another can serve as signs of cardiovascular pathology. For this reason, when choosing electrocardiographic leads the most suitable are the leads with complexes of different types. Table 1 shows the distribution of structural types according to the leads.

As may be seen from the table, type PRT is more frequently met with in leads, 2, 3, UN and UP, type PRST in leads GP-3, GP-5 and GL-3, and type PQRT in leads GP-1 and GL-1. These three types of structures are

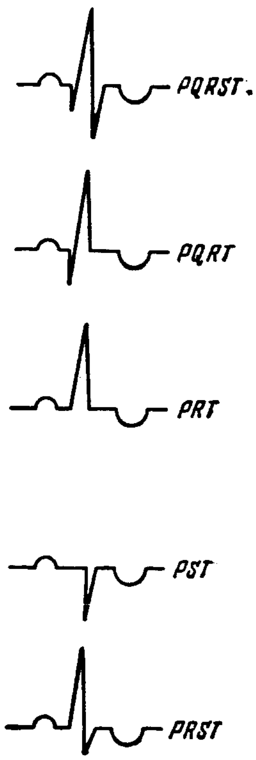


Figure 2. Structural Types of Electro-cardiographic Complexes

Table 1. Distribution of Structural Types of Electro-cardiograms in Various Leads

| Leads | Structural types of electrocardiogram | | | | |
|---|---|---|---|---|---|
| | PQRST | PQRT | PRT | PST | PRST |
| 1 | 1 | 6 | 13 | - | - |
| 2 | 4 | - | 15 | - | 1 |
| 3 | - | - | 16 | - | 4 |
| UN | 1 | - | 17 | - | 2 |
| UL | 1 | 8 | 11 | - | - |
| UP | 1 | 3 | 14 | - | - |
| GP-1 | - | 11 | 2 | - | 7 |
| GP-3 | - | 1 | 3 | - | 16 |
| GP-5 | - | - | 8 | - | 12 |
| GL-1 | 3 | 15 | - | 1 | 1 |
| GL-3 | - | 2 | 3 | - | 15 |
| GL-5 | - | - | 10 | 2 | 8 |
| GN-1 | 7 | 4 | 9 | - | - |
| GN-3 | 5 | 4 | 3 | - | 8 |
| GN-5 | - | 2 | 8 | - | 10 |

most common. Leads 2, 3, UN, UP, GP-3, GL-1 and GL-3 have the most stable structure.

In the analysis of electrocardiograms the parameters of time and systole are of paramount interest. The amplitude measurements are of lesser interest. Table 2 summarizes data on certain parameters of the electrocardiogram in dogs according to the results obtained from tracings from the standard lead 2.

Table 2. The Values of Certain Electrocardiographic Parameters in Dogs

| ECG parameters | Mean values | Limits of normal variation |
|---|---|---|
| RR, sec | 0.7 | 0.4-1.2 |
| $\Delta$RR, sec | 0.28 | 0.15-0.7 |
| $P_Q$, sec | 0.11 | 0.09-0.13 |
| QRS, sec | 0.05 | 0.03-0.08 |
| CP,% | 32 | 26-48 |
| P, mv | 0.35 | 0.2-0.5 |
| R, mv | 1.1 | 0.7-1.5 |
| T, mv | 0.3 | 0.1-0.6 |
| Electrical axis degrees | 72 | 50-85 |

When choosing the electrocardiographic leads it is necessary to consider the sharpness of the waves and the intervals of the curve, the stability of the form of the complexes, and the possibility of obtaining maximum information with the smallest number of leads. When using one lead, the most suitable will be leads 2, UN and GP-3. When two leads are used 2 and GP-1, UL and GP-3, UN and GL-1, and 3 and GL-3 can be recommended.

In the analysis of time relations PQ and ST have the greatest significance, and also $\Delta$RR. Our experiments have shown that the arrhythmia index is a particularly sensitive indicator of situations which are particularly severe for the animal. A considerable decrease in $\Delta$RR evidently indicates deterioration in the functional state of the myocardium.

Changes in the amplitude of the waves most frequently depend on changes in position of the heart in the thoracic cage. Thus, large respiratory variations in amplitude were determined in leads GL-5, GL-9 and GN-9. In order to determine the electrical position of the heart, it is convenient to use leads UL and UN. In the case of lead UL, amplitude

variations in the R wave are usually due to changes in the electrical axis of the heart at different phases of the respiratory cycle.

The suggested choice of leads and the development of a single approach to the analysis of electrocardiograms, and also individual selection of animals, are of great importance in improving the quality of scientific investigations in order further to develop space physiology.

Summary

11707

The standardization of electrocardiographic methods and their application to the study of dogs during space flights are discussed. The work is based on 500 ECG tracings obtained on twenty dogs.

It is concluded that all the ECG wave patterns may be subdivided into five fundamental structural types. Furthermore, it is suggested that the following leads are most suitable for the above purpose: 2, 3, CR-1, CR-3, CL-1, CL-3, VF and VL. The values of certain electrocardiographic parameters, including RR, ▲RP, PQ, QRS and systolic index are given, showing the limits of normal variations. All the tracings were taken on a type 4 PFD-7 electrocardiograph.

Author

p 471-482 refs N64 11708



# THE STUDY OF BIOELECTRIC POTENTIALS AND OXYGEN PRESSURE IN BRAIN TISSUES DURING HYPOXIA

Ye. A. Kovalenko and V. B. Malkin

It is now well established experimentally that changes in the electrical activity of the brain (EEG) are one of the earliest and most sensitive indications of alterations in the functional state of the central nervous system which occur during acute oxygen starvation.

Various authors have described the characteristic pattern of EEG (electroencephalography) changes which develop with the increasing hypoxia that occurs during ascent to high altitudes or during breathing gas mixtures deficient in oxygen. In the early stages of hypoxia when adaptive respiratory and circulatory reflexes occur, the EEG shows, as a rule, an increase in high frequency oscillations and, in certain cases, an increase in the amplitude of the $\alpha$-rhythm. With further increase of the hypoxic state, the EEG shows a gradual buildup of the slow rhythm; at first M-shaped $\alpha$-waves and then single oscillations of 6-7 cs appear.

With further deepening of the hypoxia, slow oscillations become more pronounced and prevalent on the EEG, their amplitude increasing steadily while their frequency decreases. This is shown by the appearance of long groups of waves with a frequency of 5-6 cs, which are gradually displaced by oscillations of 3-4 cs. The latter are followed by even longer, high amplitude oscillations (A. Davis, H. Davis, Thompson, 1938; Kornmuller, Palme, Strughold, 1942; Vorob'yev, Dzidzishvili, 1945). In experiments on man a rigorous relationship was established between the particular EEG changes and the corresponding symptoms indicating disturbance in central nervous system activity. For example, during the appearance of the $\alpha$-wave (6 oscillations per sec) the following symptoms were observed: a delay in solving arithmetical problems, an increase in the number of errors made, and clear-cut disturbances in handwriting. The prepondance of high amplitude oscillations in the 2-4 cs range of the EEG is as a rule accompanied by the appearance in the subject of serious disorders of the central nervous system which are manifested at first as local and then generalized clonic spasms, as well as inadequate appraisal by the subject of his surroundings, disturbance of consciousness and even full loss of consciousness (Shpil'berg, 1944; Kornmuller, Palme, Strughold, 1942).

Since the EEG changes reflect to a measurable degree the level of hypoxia, certain investigators have suggested using an electroencephalographic scale terminology, viz: 6 c/s hypoxia; 3 c/s hypoxia, and so forth (Noell, 1950).

Studies on animals have also shown that, with increasing hypoxia, a regular shift of the frequencies to the left takes place, i.e., a gradual preponderance of longwave oscillations on the EEG develops. Experiments on animals have also made it possible to trace the EEG changes that occur in extreme states of acute hypoxia which terminated in the animal's death. It was found that with a further buildup of hypoxia the amplitude of the longwave oscillations decreases and a full depression of the bioelectric currents gradually sets in (Gelhorn, 1948; Livanov, Parfenova, 1945; Malkin, 1960). The great interest which the physiologists have shown in the generation of longwave oscillations during hypoxia is fully understandable. It was of particular importance to discover whether this form of bioelectric activity depends on the level of the oxygen supply to the brain tissue.

In order to explain these problems, studies were undertaken in which the bioelectric potentials of the brain were recorded simultaneously with determinations of the oxygen content of the blood.

In experiments with human subjects, application of aural oxyhemometry showed that during acute oxygen starvation the EEG displayed slow oscillations which differed sufficiently according to the oxygen saturation of the arterial blood, while allowing for individual variations, for them to be significant. According to G. V. Altukhov, V. B. Malkin and I. S. Balakhovskiy (1954), slow oscillations ($\beta$-waves) appeared during blood oxygen saturations ranging from 50 to 78 percent. In the last mentioned work only the oxygen saturation of blood is correlated with clear-cut changes in the bioelectrical activity of the brain. However, it was not possible to assess the importance of local differences in blood redistribution, and so no conclusions could be made about the level of the oxygen supply to that part of the brain from which the bioelectric potentials were recorded.

Obviously, it is of great interest to study simultaneously the bioelectrical activity and the oxygen tension in the same region of cerebral tissue during the induction of hypoxia.

Investigations of this type would show whether these or other changes which occur in cerebral bioelectrical activity during hypoxia depend solely on the level of the oxygen supply to the tissues, or whether they also depend to a considerable extent on other factors, viz: changes in sensory input, or changes in activity between the individual nerve centers, etc.

It was also of interest to discover whether during the development of hypoxia there is a well-defined threshold of oxygen tension in the cerebral tissue which is necessary for maintaining the bioelectric activity of the brain. To study these problems, experiments were carried out in which polarographic determination of oxygen tension in the cerebral

that the depth of respiration could be indirectly estimated. The animals were tested for 10-15 days after the insertion of the electrodes.

Acute hypoxia was induced in the animals by making them breathe an oxygen-deficient mixture under conditions of normal atmospheric pressure. The mixture was supplied from cylinders through a reducing valve into a bag which was connected to the mask by corrugated tubing. The mask was put on the snout of the animal.

The second series of experiments was carried out in a pressure chamber. Acute hypoxia was induced by a sudden decrease in atmospheric pressure (within 1-2 sec) which was equivalent to ascent of the animal to an altitude of 12,000-20,000 meters. In this way the animals in this series of experiments were subjected to the simultaneous effect of low barometric pressure and acute oxygen starvation.

## Experimental Results

In the first series of experiments, carried out on three dogs and three rabbits, acute hypoxia was induced in the experimental animals by making them breathe a gas mixture of extremely low oxygen content (1-1.5%).

During the onset of hypoxia, all the experimental animals exhibited marked regular changes in oxygen tension in the brain tissues. Disturbance of respiration, changes in the ECG and EEG and the symptoms correponding to disturbance of the central nervous system were also noted. These changes appeared within definite time intervals which were similar for the different animals. For instance, in the experiments on the dogs it was observed that with the increased frequency and amplitude of respiratory movements there was a marked increase of pulmonary ventilation which was already noticeable within 18-20 seconds after breathing the hypoxic gas mixture. Similar respiratory changes were observed somewhat earlier in the rabbits, viz: within 12-15 seconds.

Regular changes in the EEG were observed in the experimental animals during the development of hypoxia. These could be conveniently divided into two phases.

The first phase was characterized by the appearance of high voltage slow oscillations of gradually increasing amplitude. As the hypoxia deepened, the amplitude of the slow oscillations decreased. Their frequency continued to decrease and a gradual depression of the bioelectric currents developed.

In the second phase, the depression of bioelectric currents was characterized by a sharp decrease in the current amplitude and separate

tissues was used, a technique adapted by Ye. A. Kovalenko to the conditions of the pressure chamber experiment. These studies were run in parallel with the encephalographic observations.

## Materials and Methods

The experiments were carried out on dogs and rabbits which prior to the experiment had platinum electrodes inserted into the motor cerebral cortex and into the thalamus. The electrodes which were mounted in a perspex holder were fixed to the skull by means of dental cement. A polarograph was assembled for the determination of the oxygen tension in the brain. It consisted of current source, potentiometer, voltmeter and two high sensitivity galvanometers with a series of shunts. Special clips with a silver chloride tip were used as anode electrode. Oxygen tension was determined in the cerebral cortex and sub-cortical layers of the brain from the galvanometer readings. Graphic recording of galvanometer readings on a photokimograph tape were carried out during one part of the experiments.

Oxygen tension was determined from the magnitude of the "limit current" in relative units. Oxygen tension in brain tissues during respiration with air at ground level was taken as 100 percent.[1]

Simultaneously with the polarographic record which was carried out on on a six channel pen oscillograph, the animal's EEG, ECG and chest respiratory movements were recorded.

An EEG lead was taken from the same electrodes which were used for the polarographic record. In order to eliminate artifacts and interference, all the conductors were carefully screened and the animal itself was grounded. The 0.6 V d.c. from the polarographic installation did not impede the EEG recording.

The electrocardiogram was made using needle electrodes with leads from the right front and left hind paws (second standard lead).

Respiration was registered by means of a carbon sensor incorporated in a bridge circuit. This method made it possible to record the frequency and rhythm of respiratory movements with sufficient accuracy so

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[1]Detailed description of the method for polarographic determination of oxygen tension in brain tissues is given in the journal "Patologicheskaya Fiziologiya i eksperimental'naya terapiya", No. 2, 1961.

slow oscillations appeared in the EEG only periodically. These, in a number of cases, coincided with the terminal respiratory movements.

It is of interest that the sequence of functional changes that occurred with hypoxia was relatively constant. This may be related in the first instance to the changes occurring in the bioelectric activity of the brain. Thus, distinct changes in the EEG (viz: the appearance of high voltage slow oscillations during the first phase) were observed within 20-25 seconds after the onset of respiration with the gas mixture. The duration of the first phase in various animals was from 8-10 to 20-30 seconds, after which the second phase began, namely, the depression of bioelectric currents. It is difficult to be precise about the duration of this phase since, if the animals were not given atmospheric air or oxygen to breathe after the experiment, the depression of the bioelectric currents on the EEG was found to be similar to that found in the state of clinical death. During the period of terminal breathing, if the animals were within a certain time switched over to breathing atmospheric air or oxygen, a gradual restoration of bioelectric activity took place. At the same time, during this recovery period the same changes in the EEG were observed as took place during development of the hypoxic state. The phase of slow oscillations appeared again during the recovery period. However, this phase was more prolonged than that which occurred during the development of the hypoxic state, and was followed by a return of the normal EEG.

Simultaneously with these changes in the EEG, regular changes in the ECG were observed which are characteristic of the various phases of the changes occurring in cardiac activity during hypoxia. If a very rapid oxygen starvation occurred in these experiments, the initial changes in cardiac activity (development of sinus tachycardia) could not always be detected. Slowing of cardiac activity with corresponding changes in the ECG was detected in various animals within 30-45 seconds, after which, at varying times, the development of sinus tachycardia was observed, preceded by paralysis or very deep inhibition of the vagal centers. This phase was of relatively short duration and was followed by gradual slowing of the cardiac rhythm accompanied by changes in the ECG characteristic of the onset of death.

In a number of animals, the relative decrease of oxygen tension in the brain tissues during development of the first and second phases of the EEG changes associated with hypoxia varied within a considerable range. For instance, in certain experiments, the appearance of the slow oscillations on the EEG was observed during a relatively small drop in the oxygen tension in the brain tissues, by only 20-25 percent from the original figure. At the same time, in other animals, these changes in the EEG were observed during a much more pronounced drop in the oxygen tension of the brain tissues.

The great range in the level of the fluctuations in the oxygen tension of the brain tissues found in different animals was also noticeable on the EEG changes which developed during the second phase of hypoxia. Characteristic changes in the EEG, ECG, respiration, and oxygen tensions of the cortex and sub-cortical layers are shown in Figure 1.

It was of great interest to establish whether the relationships between the EEG variations occurring in the same animal are constant and depend on the level of the oxygen tension in the tissues of the brain. In fact, is there a rigorous relationship between the appearance of slow oscillations and depression of bioelectric currents on the one hand and the level of oxygen tension in the tissues of the brain on the other?

In order to answer this question, the levels of oxygen tension in brain tissues were compared in one and the same animal during the appearance of slow oscillations in the EEG at the corresponding stage of hypoxia and during the recovery period. In addition, it was also necessary to compare the level of oxygen tension with the onset of the slow oscillations on the EEG of one and the same animal, during the ascent to various altitudes.

The results of these experiments did not reveal the existence of a rigorous relationship between the appearance of changes in the EEG, and the levels of oxygen tension in the brain tissues during hypoxia, during the post-hypoxic period, and during the ascent of animals to various altitudes. For instance, in one experiment the appearance of slow oscillations was noticed during an ascent by the animal to 7 km, when the oxygen tension of the cerebral cortex was depressed to 46 percent and that in the sub-cortical layers 44 percent. The slow oscillation phase was observed in the same animal when the oxygen pressure in the cortex was depressed to 20-46 percent of the original value, and in the sub-cortical layers from 28 to 44 percent. When the oxygen tension in the cortex was decreased to 16-20 percent, and in the sub-cortical layers to 22-28 percent, a depression of the bioelectric currents was observed with the simultaneous appearance of separate slow oscillations imposed on the background of the terminal respiration and ventricular bradycardia.

Recovery of bioelectric activity, i.e., the appearance of slow oscillations, was observed in this experiment when the oxygen tension in the cortex of the brain rose to 35 to 40 percent of normal, and in the sub-cortical layers between 45 to 50 percent of normal, i.e., levels somewhat higher than those at which the slow oscillations appeared on the EEG during the development of the hypoxic state.

At ground level, during almost full restoration of oxygen tension in the brain cortex (84 to 90 percent) and an increase in the level of the

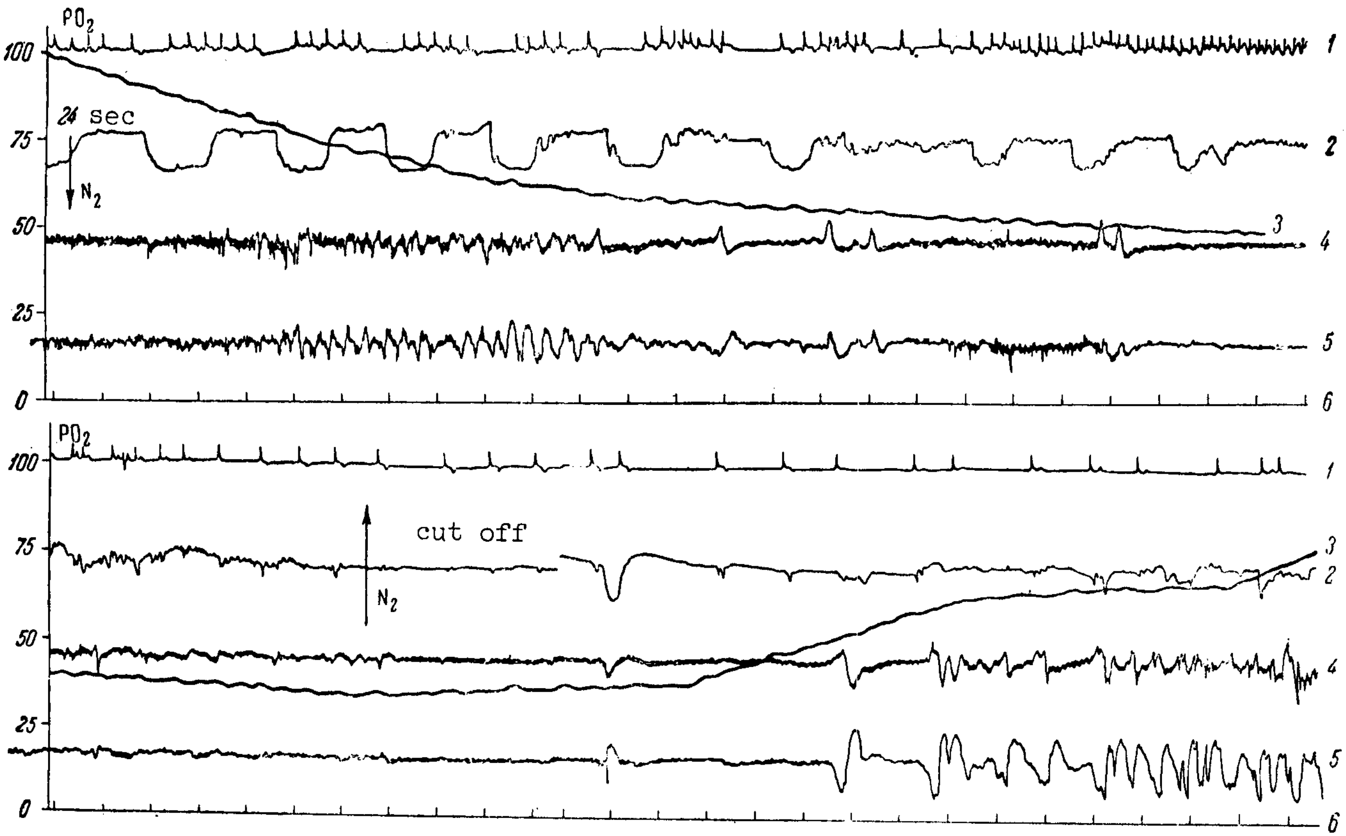


Figure 1. Experiment on Dogs. Variations in the EEG, Respiration, ECG and Oxygen Tension in the Tissues of the Brain during Respiration with a Gas Mixture Extremely Poor in Oxygen, $O_2$ 2%, $N_2$ 98%

1 - ECG; 2 - Respiration; 3 - Oxygen Tension in the Brain Cortex (in % of the Original Value); 4 - EEG of the Brain Cortex (Unipolar Record); 5 - EEG of the Sub-cortical Brain (Unipolar Record); 6 - Time Marker in Seconds. Ordinate - Partial Pressure of Oxygen in the Cortex of the Brain (in %); Abscissa - Time in Seconds. Registration Started upon 24 sec of Breathing with Nitrogen

oxygen tension in the sub-cortical formations up to 120 percent[1] (based on the original control record), the EEG differed principally by the brief appearance of high frequency oscillations (Figure 2).

The definite scatter found in different animals in the level of oxygen tension in their brain tissues at which non-ambiguous changes in the EEG due to hypoxia are observed may, in the first instance, be caused by differences in the location of the electrodes in the brain tissue relative to the surrounding blood vessels, and the nature of the reactive changes of the tissue around the electrode itself, differing with the experimental animal. In view of this, a strict consistency in the oxygen-tension levels in different animals was not to be expected. At the same time, the relative changes in the oxygen tension of the brain tissue found in one and the same animal (where there were constant conditions determining the flow of the biocurrents and of oxygen diffusion towards the electrode) enabled the changes of the biocurrents in relation to the oxygen tension measured in the tissues to be assessed.

After rapid ascent of the same animals to the altitudes 15, 17 and 20 km, the appearance on the EEG of high voltage slow oscillations was noticed within 12-18 seconds. The appearance of a fully developed biocurrent depression occurred within 18-30 seconds. As a rule, with increasing altitude, the differences in time of the appearance of the high voltage, slow oscillations and the subsequent depression of the bioelectric currents was altogether 3-5 seconds. This is clearly connected with the somewhat higher rate of blood and tissue deoxygenation occurring in the animals during increasing rarefaction.

Oxygen tension in the tissues of the brain in these cases is extremely low, of the order of 15 to 30 percent of the original value, and the difference in the level of oxygen tension during the appearance of the σ-rhythm and the depression of bioelectric currents was insignificant. Thus it may be said that when the oxygen tension in tissues during ascents of the order of 12,000 m is compared with levels in tissues from ascents to 20,000 m, the σ-rhythm appears at the higher altitudes, where a lower oxygen pressure occurs, against the background of a steeper curve of oxygen pressure in the brain tissues (Figure 3).

The experimental results show that there is a strict relation between the changes in the bioelectric current in the brain and the disorders of the central nervous system, respiratory and cardiac systems induced by hypoxia. At the same time, it was not possible to detect a close

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[1] The increase of oxygen pressure above the original 100 percent level is caused apparently by the development of post-hypoxic hyperamia.

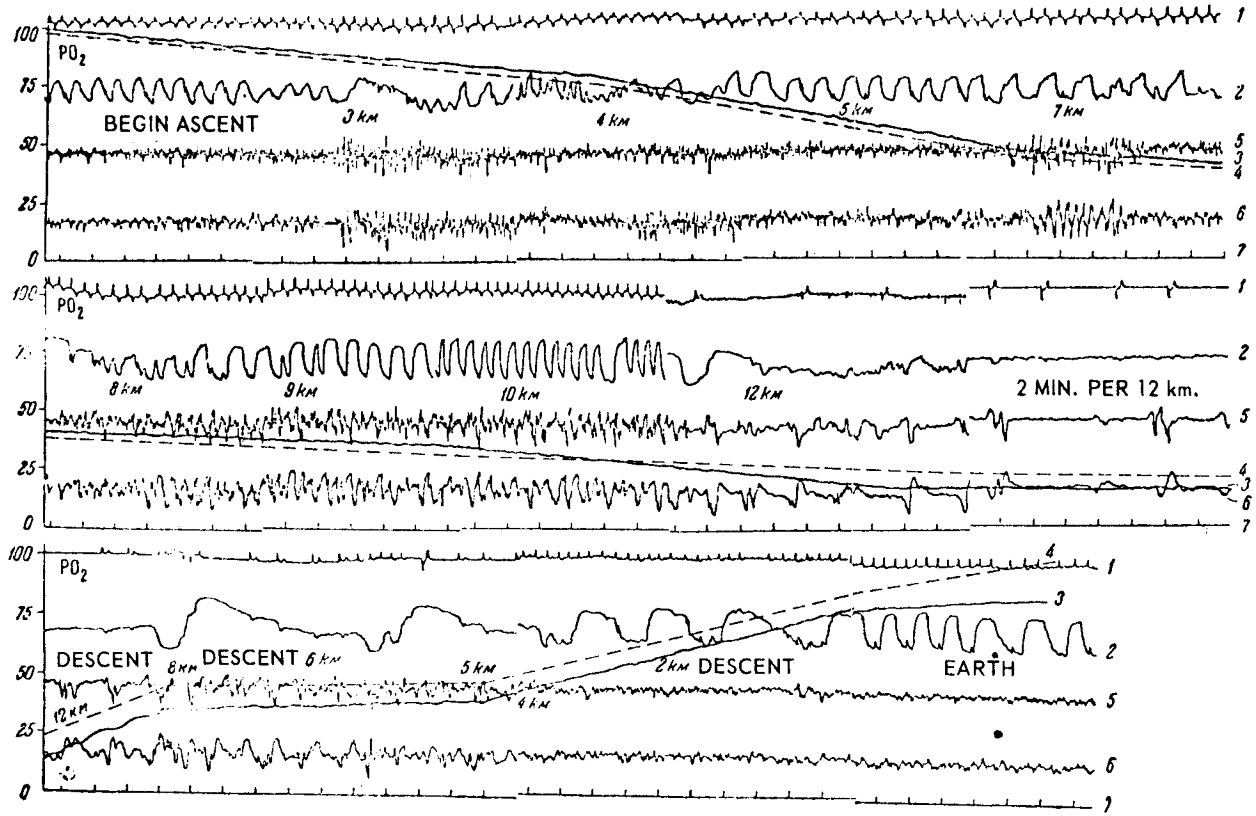


Figure 2. Experiment on Dog. Variations in EEG, Respiration, ECG and Oxygen Tension in the Cortex and Sub-cortical Brain during Ascent to an Altitude of 12,000 Meters with Oxygen

1 - ECG; 2 - Respiration; 3 - Oxygen Tension in the Cortex (Full Line); 4 - Oxygen Tension in the Sub-cortical Layers (Broken Line); 5 - EEG of the Cortex (Unipolar Record); 6 - EEG of the Sub-cortical Region (Unipolar Record); 7 - Time Marker, in Seconds

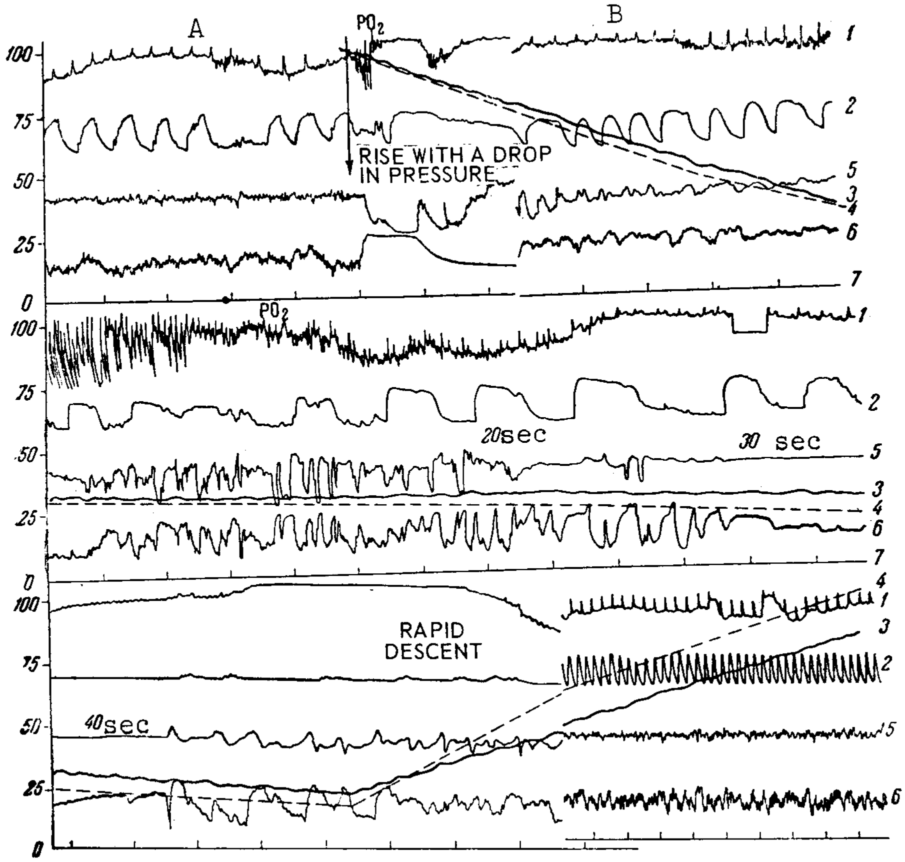


Figure 3. Experiment on Dog. Variations of EEG, Respiration, ECG and Oxygen Tension in the Cortex and Sub-cortical Layers of Brain during Rapid Ascent (Fall of Pressure) to the Altitude of 20 km

1 - ECG; 2 - Respiration; 3 - Oxygen Tension in the Brain Cortex (Full Line); 4 - Oxygen Tension in the Sub-cortical Brain (Broken Line); 5 - EEG Brain Cortex (Unipolar Register); 6 - EEG of the Sub-cortical Region (Unipolar Register); 7 - Time Marker, in Seconds; A - Normal; B - After Drop

relation between the level of oxygen tension determined by the polarographic methods and the various phases of the EEG changes occurring during hypoxia. Changes of the EEG - σ-rhythm and depression in the bioelectric activity - at high altitudes (viz: 17-20 km) due to hypoxia appeared at a considerably lower oxygen tension in the tissues of the brain, than similar EEG changes occurring at lower altitudes in the same animals (12 km) which took place at much higher levels of oxygen tension. From this it may be asserted that the functional state of the central nervous system

during the development of acute oxygen starvation is determined not only by the extent of the lowering of the oxygen tension, but also by the time parameters characteristic for the development of this process.

Summary

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Investigation of oxygen tension and of bioelectrical activity of brain tissues was carried out on dogs and rabbits during acute hypoxia. ECG and respiratory rates were also recorded.

Under conditions of hypoxia the animals tested revealed regular changes in their EEG which could be divided into two phases:

The first phase was characterized by the appearance of high voltage slow waves of gradually increasing amplitude, and the second phase developing with the hypoxia was characterized by a depression of biocurrents and a sharp diminishing of the amplitude and frequency of biopotentials.

The results of the above experiments in various animals did not show a strict correlation between the changes in EEG and oxygen tension which occurred during the development of hypoxia and after it. At the same time relative changes in oxygen tension in the brain tissue of animals give grounds for believing that biopotential changes depend upon the oxygen tension in tissues.

It should be noted that hypoxial changes in EEG depend not only upon a decrease in oxygen tension in a given part of the brain, but also upon the duration this decrease was being observed.

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# COORDINATION OF ARBITRARY MOVEMENTS OF A HUMAN BEING IN A CHANGING GRAVITATIONAL FIELD

L. V. Chkhaidze

Certain problems connected with the coordination of human movements in a modified and, in particular, an increased gravitational field have been studied by various authors (Vinokurov, Levashev, Khromushkin, 1946; Babushkin, Isakov, Malkin, Usachev, 1958; Usachev, 1961; and Geratewohl, et al., 1956). However, the author of this paper studied these phenomena from the point of view of the general theory of control and control systems which have recently become of considerable importance. As the earlier workers had largely ignored these problems, he aimed at accumulating a large amount of material so that the variational statistical authenticity of the average values could be determined and the corresponding mathematical relations of the possible disturbances of the motor activities under study established.

According to contemporary ideas, the control of motor activity (if by the latter is understood an arbitrary movement containing both sensory information and information about specific muscle actions) may be broken down into the activities of two more or less well defined closed-control circuits--external and internal (Chkhaidze, 1960). The external circuit controls the sensory element concerned in motor activity while the internal circuit controls the specific muscle actions. Each circuit consists of a programming mechanism (for the external circuit this is known as the "master mechanism"), feedback line and a collating mechanism. The programming mechanism of the external circuit is placed at a higher level in the nervous system than the internal ones. This makes the activity of the internal circuit subordinate to the external circuit (Figure 1).

Direct coupling of both control circuits is common and consists of a portion of the spinal cord, centrifugal axons, neuromuscular synapses and muscles (which, for convenience, will be called the "servomechanism"). In other words, excitation of the peripheral musculature is possible both directly through the "master mechanism" and through the programming mechanism of the internal circuit (Figure 1).

The receptor cells of all the external sense organs act as the sensors of the external-circuit feedback, while the proprioceptive pathways make up the internal circuits. They carry specific information about the state of muscle tone and the position of the parts of the body in space.

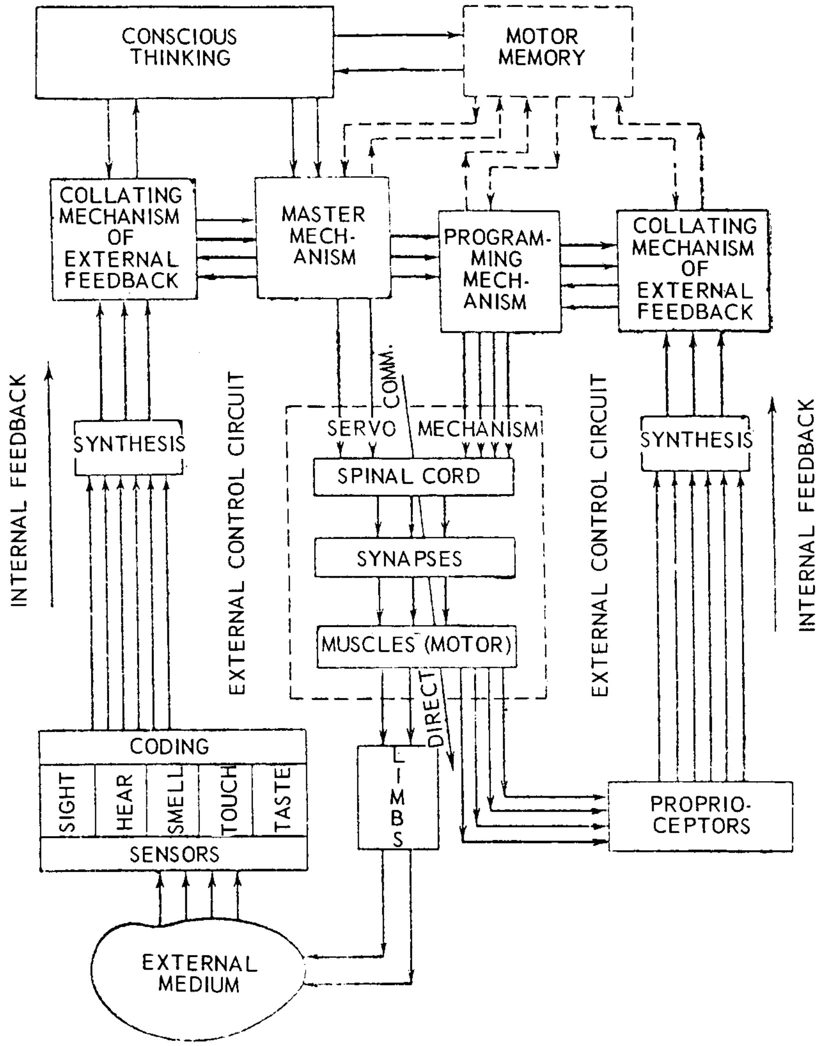


Figure 1. Probable Diagram of the Passage of Information During Coordination of Arbitrary Movements in a Man

The collating mechanism of this circuit, which is of greatest importance for the correct coordination of movement, is apparently localized in the cerebellum (Wiener, 1958).

Control of mastered arbitrary movement may be reduced to the fact that the external circuit controls the sensory side and the internal circuit controls the sequence of specific muscle contractions. According to the information supplied from the periphery and after this has been collated with the program, both collating mechanisms continuously introduce the necessary corrections for the right course of movement. The corrections are introduced through the programming mechanisms into the corresponding synergic parts of movement.

If the above described correcting mechanism becomes disturbed, movement loses its dynamic stability through incoordination of parts of the limbs. This is due to volleys of nerve impulses travelling along wrong nervous pathways and so causing this lack of coordination.

A similar phenomenon is observed (apart from pathological cases) during mastering of an unknown motor habit. Thereby, the appropriate mechanism of the internal circuit will not have a detailed program for controlling the given movement and the control of the performance of some of the details of this movement will be taken over by the external circuit, using to a major extent programs of similar habits developed earlier in the internal circuit.

However, when certain movements are repeated many times, the proprioceptors of the peripheral muscles interact with the internal circuit and by means of continuous ("irradiation") excitation of the nerve centers form appropriate conditioned reflex arcs (couplings) and the detailed program for correct execution of the movement is thus evolved. Simultaneously, the collating mechanisms of the internal circuit begin to operate. Then, control of the movement with appropriate distribution of the functions between the circuits becomes possible.

The extent to which the coordination between movements will occur depends principally on the activity of the internal circuit. Taking this as the starting point, we have chosen during the planning of the experiment a habit which excludes as far as possible the activity of the external circuit. The subject should develop a habit perfectly, so that its execution in an increased gravitational field should affect only the information derived from the proprioceptors. In this way, only this could change the conditions of the collation mechanisms of the internal circuit controlling the given movement. For this reason, a movement with a well developed set of basic dynamic components was selected, i.e., attempts to press a finger against a hard object. It is not difficult to see that disturbances in the coordination of such a movement are predominantly expressed in distorted single attempts, since during increased gravity the increased weight of the limbs will add to the force applied (Geratewohl, 1960). Clearly, this will immediately distort all the information coming from the proprioceptors and so disturb the activity of the collating mechanisms of the internal circuit. Since the amount of each exertion can be most accurately assessed in studies of this type of motor habit, which are of a clear cut nature, we decided to determine the ability of the subjects to differentiate between the amount of exertion of two pressures, one following the other under conditions of overloading. The subject was required to carry out the above task in such a way that the magnitude of the exertion of the second pressure was equal to half the exertions of the first. Each pressure application took approximately one second.

The quality of the accomplishment of the motor habit was determined by the relationship between the first and the second exertion during pressings. As a measure of the quality, and consequently of coordination, of a given movement, the relationship of the difference between the exertions of the first attempt to the magnitude of the second attempt was chosen. This relation was called the index of differential exertion and was denoted by the letter F. It is clear that when the subject apportioned his exertions to give a ratio equal to two, then F = 1. In the opposite case, this indicator decreased and became equal to zero when the magnitude of the exertion of the second pressing was equal to the magnitude of the first one.

Before carrying out the fundamental observations, all the subjects underwent special training so as to develop the above habit in a normal gravitational field. During this training the subjects carried out not less than 8000 pairs of pressings during an average 150 minutes of actual working time. Only during the first exercises were the subjects able to control visually the magnitude of the pressings. As the habit became more or less acquired and F reached on an average 0.6, visual control was withdrawn and the subjects acquainted themselves with the habit only by means of the proprioceptive sensations. The quality of the task was controlled only by the experimenter who followed the progress of the training on recording instruments. Training was complete when the indicator F reached a predetermined limit of 0.85-0.90 and then stablized.

The main experiment was carried out immediately afterwards and consisted of 2 minute long tensometric recording of the above habit in conditions of 3 and 7 units of overload, and also during the overloads increasing within 2 minutes from 0 to 7 units. Before experiments began at least 50 pairs of pressings were recorded under normal gravitational field conditions.

Figure 2 shows a sample of the record of the learned movement in normal and increasing gravitational fields.

The subjects were subjected to gravitational overloading by means of rotation in the chair of a centrifuge. Since certain directions of

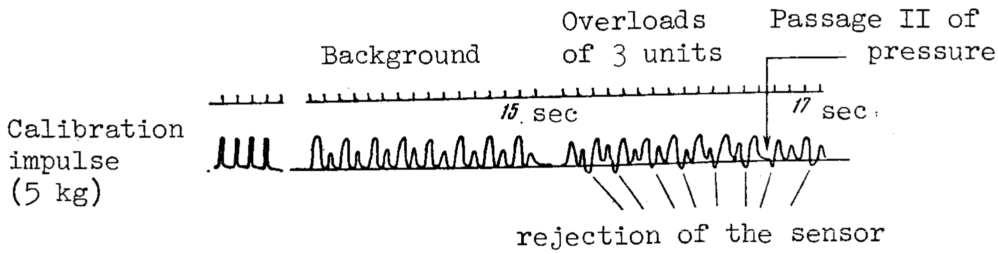


Figure 2. Record of Several Pairs of Pressings in a Normal (Background) and Increased Gravitational Field (Overload of 3 Units)

overloading may impair blood circulation in the brain and so independently affect the coordinated movements irrespective of the magnitude of the gravitational field (Usachev, 1961), the acceleration vector followed the chest-spine line, and so prevented disturbances in the blood circulation of the upper parts of the body. This was shown by means of additional observations which were carried out simultaneously with the other readings.

Recordings of action potentials of the muscles of the forearm, which play a leading part in the movement under consideration, were also carried out in certain experiments. This determined the extent and timing of their contraction under differing conditions of overload. They verified the relations found earlier (Figure 4).

Altogether 24 experiments were carried out with four subjects carrying out not less than 50 pairs of pressings during each overload.

The processing of the material collected was reduced to determining the average values of the pressures, the square of the deviations, evaluation of the differentiation index of the whole as well as at 20 second intervals during every experiment, evaluation of the coefficients of correlation and regression, and also the magnitude of the error according to conventional rules.

We shall consider the table which contains the average data of these indices.

The average magnitude of the exertion during the first pressing ($M_1$) for all types of overload remains at one and the same level taking into consideration the magnitude of the deviation. The average of the exertion of the second pressing ($M_2$) shows an increase according to the magnitude of the overload even when the possible deviations are taken into account.

The magnitude of the square of the average deviations ($\sigma$) for the exertions of the second pressings are greater than those of the first and, although in both cases they are higher during gravitational overloading than in a normal gravitational field, the magnitude of the overload affects the limits of these deviations very little.

The magnitude of the error of the averages (m) confirms the reliability of the data obtained and comparison of the series is excellent.

The data recording the correlation coefficients and regression coefficients are not stable. On the contrary, the index of differentiation exertions which we have introduced illustrates the disturbance in the

motor habit which was executed very well. It follows from this table that with increasing gravitational overload the differential index, which under normal gravitational field conditions has an average value of approximately 0.80, always decreases.

AVERAGE DATA ACCORDING TO ALL THE INDICES

| Index | Without overload (background) | During stable overload | | During increasing overload up to 7 units |
|---|---|---|---|---|
| | | 3 units | 7 units | |
| **Average value of exertions, grams** | | | | |
| I pressing ($M_1$) | 5353 | 5291 | 5408 | 5410 |
| II pressing ($M_2$) | 2499 | 3290 | 3383 | 3291 |
| **Average quadratic deviations, grams** | | | | |
| I pressing ($\sigma_1$) | ±516 | ±689 | ±657 | ±630 |
| II pressing ($\sigma_2$) | ±446 | ±861 | ±872 | ±687 |
| **Error of the average, grams** | | | | |
| I pressing ($m_1$) | ± 75 | ± 98 | ± 98 | ± 87 |
| II pressing ($m_2$) | ± 63 | ±121 | ±131 | ±102 |
| Correlation coefficient between the exertions of the pressings | 0.40 | 0.50 | 0.34 | 0.45 |
| Coefficient of regression of the exertions between the second and first pressings | 0.35 | 0.63 | 0.42 | 0.48 |
| Correlation coefficient error ($m_k$) | 0.09 | 0.07 | 0.10 | 0.10 |
| **Index of exertion differentiation (F)** | | | | |
| Average value during the time of overload | 0.83 | 0.63 | 0.57 | 0.6 |
| At the beginning of the overload | 0.81 | 0.53 | 0.41 | 0.70 |
| In the middle of the overload period | 0.84 | 0.68 | 0.59 | 3<br>0.57 |
| At the end of the overload period | 0.82 | 0.73 | 0.66 | 0.52 |

In other words, with an increasing gravitational field the ability of our subjects to differentiate between exertions gets worse and, therefore, the second pressings are approaching the first ones with regard to the degree of exertion.

The graph, Figure 3, gives the values of the differential index of exertions (F) along the ordinate axis and the magnitude of the gravitational field (g) on a logarithmic scale. It can be seen from this graph that the relation between the differentiation index and the logarithm of the gravitational force is, in the case of constant overload (straight line I), represented by a straight line.

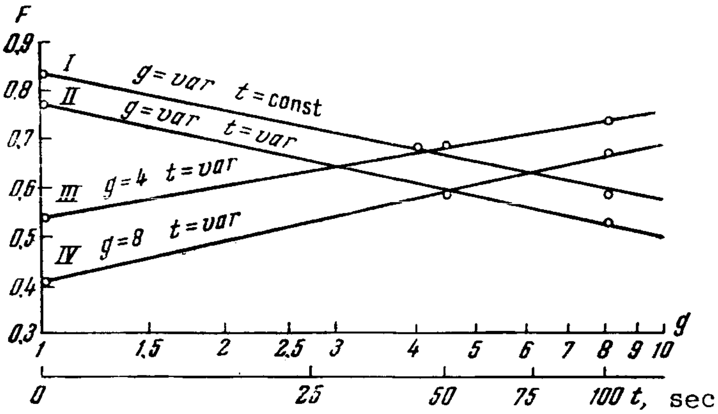


Figure 3. Relation Between the Coefficient of Differentiation of the Exertions (F), Logarithm of the Exertion Due to the Gravitational Force (g) and the Time of Execution of the Pressings (t) in Various Gravitational Fields

I - Average during a stable field of 1, 4, 8 g (lasting for a period of 2 min);
II - during a field increasing from 1 to 8 g within the same interval of time;
III - changes during a 2 min execution of pressings in a field of 4 g (3 g overload);
IV - the same but in a field of 8 g (overload of 7 g). Data averaged from all the experiments.

This allows us to assume that the magnitude of the deterioration in the differentiation of the exertions, i.e., the real degree of disturbance in the activity of the collating mechanisms of the internal circuit controlling the given motions ($\Delta F$) is determined by the equation

$$\Delta F = \Delta y = y_0 - y$$

where $\Delta y$ - magnitude of the change in the index of the exertion differentiation (F); $y_0$ - its value in a normal gravitational field; y - in an

increased field. $\Delta y$ may in turn be represented as $\Delta y = \tan \alpha \cdot \log x$, where $\alpha$ is the angle of the slope the line makes with the abscissa axis; x - magnitude of the gravitational field.

Since $\tan \alpha$ is constant for each human being and, as may be easily seen, depends on his state and training, its value is conveniently denoted in the form of a coefficient K, which gives the equation the following form

$$\Delta y = K \cdot \log_e x \tag{1}$$

From this equation it follows that the magnitude of a possible disturbance in the differentiation of exertions, and consequently in the coordination of movements, depends in the case of an increased gravitational field, on the state of the human being placed in this field, and the logarithm of gravity acceleration.

Experiments with variable gravitational overloading (Figure 3, straight line II) showed that this relation is retained also in the case when the gravitational field increases uniformly from 1-8 g. Further analysis of this table and Figure 3 showed that during systematic accomplishment of the movement in conditions of constant overload, the index of exertion differentiation does not remain at its low original level but increases and tends to the normal. It is represented on the graph by lines III and IV for the overloads of 3 and 7 units, respectively. Time, which is plotted along the abscissa axis carrying the overload scale, appears to be important in this case. It follows from these data that in our experiments the relation between the indices of differentiation and the logarithm of the time required to accomplish the movement in an increased gravitational field may also be satisfactorily represented by a straight line. Consequently, it is possible to assert that the improvement in the index of the differentiation of exertions, i.e., the real coordination of the movements learned is expressed by a similar equation to (1)

$$\Delta y = K \log_e t \tag{2}$$

where K is the coefficient defining the state of the human being placed in the increased gravitational field, which is also expressed by $\tan \alpha$; t is the time during which the movement made is accomplished; $\Delta y$ - magnitude of restoration of the basic dynamic components of the movement.

However, it does not follow that during the prolonged exposure of a human being to an increased gravitational field the improvement in the quality of accomplishing the basic components of movement (in our

experiments the index of exertion differentiation) will increase as much as is desired. It is clear that the relation quoted above is preserved only within definite limits, for instance, before the indicators return to a known norm. For that reason we merely state the fact that the restoration of the index of exertion differentiation during gravitational overloading of 3 units takes place in much easier conditions than during the overload of 7 units (this is clear since in Figure 3, line III, which illustrates the course of coefficient F, restoration during overload of 3 units is higher than line IV for the overload of 7 units).

It follows from equation (2) that during the systematic accomplishment of the determined motor habit under the increased gravitational field, the original deterioration in motor coordination should change with the restoration of the leading values of the dynamic indices of this movement, and also the duration of the latter should depend on the state of the subject placed in a given field, the magnitude of gravitation and the time taken to accomplish the habit.

Generalizing equations (1) and (2) by the approximation expressed by straight lines III and IV of Figure 3, it may be said that when a human being is placed in an increased gravitational field, at first, the coordination of his movements deteriorates and then, if the magnitude of the field remains at a definite level and does not increase, the dynamic components of the habit under study return to the normal, provided the movements are executed continuously in a given gravitational field.

To conclude the analysis of these data it should be said that the mathematical relations which were deduced were related to the generally known law of Weber-Fechner (Bykov et al., 1955), which to a certain extent confirms their authenticity.

Studies of action potentials of the forearm muscles carried out in certain experiments showed that overloading always caused an increase of the amplitude of the action potentials depending on the magnitude of the gravitational field (Figure 4). This not only contradicts the data obtained earlier by V. V. Usachev (1958, 1961) but the fact of the increased activity of this group of muscles also confirms our thesis that the magnitude of the pressure exertion is added many times as the apparent weight of the limbs increases. Undoubtedly, this immediately distorts the information passing back to the collating mechanism of the internal feedback with all the consequences outlined.

There are definite reasons for extrapolating the relations obtained to the zero gravitational field, i.e., into the region of weightlessness. This follows from the fact that, firstly, as can be seen from equation (2), the collating mechanisms of the internal circuit controlling the habit do not remain indifferent to the disturbances in the motor coordination and tend to bring it into a dynamically stable state; secondly, as was shown

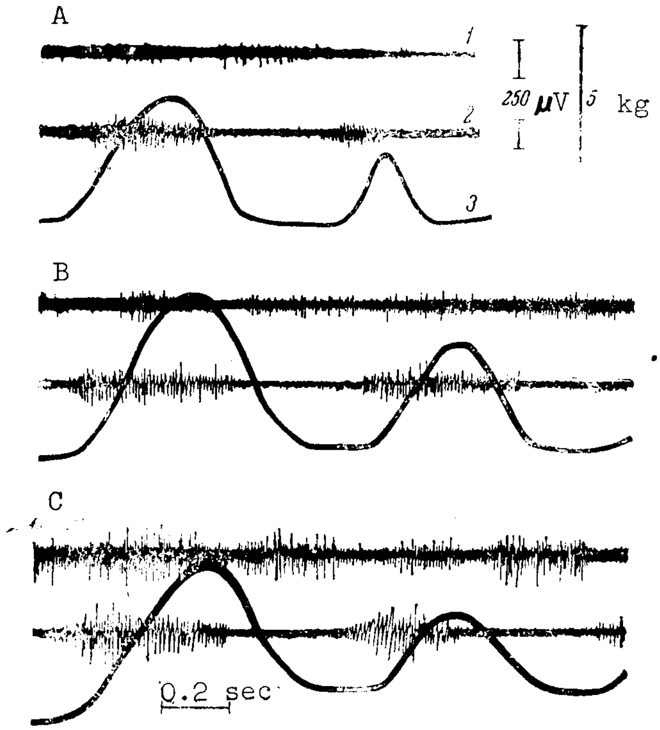


Figure 4. Oscillograph Record of Exertions of Two Pressings Following Each Other by the Finger of a Hand and of Action Potentials of the Muscles in the Forearm in a Normal Gravitational Field (A); and during 3 g Overload (B) and during Overload of 7 g (C)

1 - Action potentials in the forearm muscle; 2 - Action Potentials in the radial flexor of the hand; 3 - Tensogram of pressure efforts.

in Figure 3, the smaller the gravitational overload the weaker are the changes in the leading dynamic components of the movements, and the faster their restoration. In a normal gravitational field, as can be seen in the table, they remain generally at one and the same level. During weightlessness, the manifold increased weight of the limbs will not be added to the magnitude of the exertions as in the case of overloads and, on the contrary, their weight will not manifest itself at all. Hence, it is possible to assume that the conditions for the internal motor control circuit activity will prove if not easier at least not more difficult than in the case of overloads. Consequently, if during weightlessness the coordination of movements of a man are disturbed to a certain extent (due to changes in the information input), then this should restore itself within a certain period of time. In addition to certain

facts mentioned by Soviet astronauts, this was also confirmed in the experiments of M. A. Cherepakhin, who adhered very carefully to all the conditions of our method, recording the differentiated pressings with the finger of a hand against a hard resistance under conditions of complete, short-lasting, weightlessness.

As can be seen from Figure 5, the subjects, who at the beginning of the experiments lost the required motor coordination, regained it very quickly.

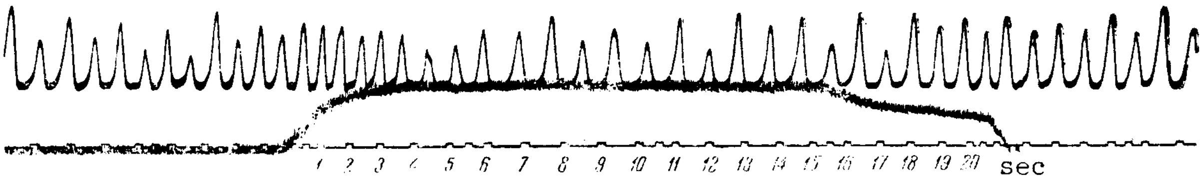


Figure 5. Recording of Accomplishment of the Differential Pressings with the Finger of a Hand against Hard Resistance under Conditions of Full Weightlessness, According to the Method and under Conditions Similar to those of Figure 2

The passage of the thick line into the upper position corresponds to the absence of the gravitational force. Experiments of M. A. Cherepakhin.

The coefficient K, which enters into the main equation, merits considerable study. It is obvious that the greater this coefficient, the higher will be the resistance of the collating mechanisms of the internal circuit, controlling movement, to changes in the gravitational field. This in its turn should depend on the general state and training of the human being under examination and may certainly serve both as a test for the selection of suitable personnel and as a method for checking the progress of training.

## Conclusions

1. The application of a general theory of control and control systems to the study of coordination of arbitrary movements in a variable gravitational field are considered as justified.

2. Coordination of arbitrary movements of a human being in an increased gravitational field shows signs of disturbance. The limits of these disturbances (insofar as the magnitude and the significance of the leading dynamic components of motion may be determined and measured) depend on the state and training of the person in the increased gravitational field, and the logarithm of the gravitational acceleration.

3. Systematic accomplishment of a disturbed motor habit in an increased gravitational field leads to restoration of motor coordination, i.e., to approximation of motor parameters and their value in the normal field. The duration of the above restoration will depend on the state and training of the person placed in this field, the magnitudes of the gravitation and the time of accomplishment of the movements only if the magnitude of the field will not increase progressively and the movements be accomplished with constant frequency.

4. Apparently, there are definite reasons for extrapolating these conclusions to the zero gravitational field, i.e., to the condition of weightlessness.

5. The above statements offer the possibility to define the muscular activity of people exposed to the action of a varying gravitational field, in particular during space flights and, consequently, may serve as initial material for the development of general problems in the special preparation of an astronaut, for instance, the training of motor habits.

6. The methods described for investigating the disturbances in the motor coordination and durations required for its restoration may serve as a test for selecting corresponding personnel and controlling their training.

Summary

11709

The author has studied the effect of increased gravitational fields under a recently acquired motor ability. Volunteers were trained to exert paired finger pressures in such a way that the first pressure of each pair was twice the force of the second. The ratio F of the difference between the pressures and the magnitude of the second pressure was used as a criterion of performance. Training was carried out first under visual instrumental control until a value of F of 0.6 was attained, after which the subject had to carry out further practice under control of his own proprioceptive mechanisms until a stable performance level of 0.85 to 0.90 was attained. The performance was then recorded by means of a tensiometer immediately before and during exposure to an acceleration of 3 g and 7 g and to an acceleration increasing gradually up to 7 g; 24 experiments were carried out on four volunteers. A mean initial value of F of 0.83 was recorded; during exposure to 3 g this fell to 0.63 and to 0.57 on exposure to 7 g. The absolute value of the first pressure was not greatly affected by acceleration and the fall in F was due to a rise in the second pressure. The results show that in an increased gravitational field subjective assessment of force is impaired. When the duration of the task was increased, however, the value of F rose again, the extent of the return to normal depending upon the state of training and other characteristics of the experimental subject.

Author

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# A METHOD OF FASTENING ELECTRODES FOR REGISTERING AN ELECTROCARDIOGRAM DURING MANNED SPACE FLIGHTS

N. A. Agadzhanyan, I. T. Akulinichev
K. P. Zazykin and D. G. Maksimov

A prolonged and reliable monitoring of the working of the heart during manned space flight is possible only if there is a permanent contact between the electrodes and the surface of the body. For that reason, the reliable fastening of electrodes is of great importance since, during the flight, the astronaut is in a special pressurized space suit and any repeated treatment of the skin or positioning of the electrodes is, for practical reasons, out of the question.

Specially designed cup electrodes have been described for use in studying the physiology of athletes. This type of electrode insures prolonged contact with the surface of the human body (Timofeyev, Antselevich, 1960; Matov, 1960). However, these electrodes are not completely satisfactory since they protrude over the surface of the body, and this results in their breaking off and, consequently, loss of the signal being transmitted.

Provision of electric contact with the surface of the human body through a conducting glue is not possible at present, since the formulations which have been used set up an irritation of the skin within a few hours (Chudnovskiy, 1961).

For that reason, the problem of developing suitable methods is of particular importance; these methods must not cause skin irritation even after prolonged contact with the electrodes.

If the astronaut's skin in the region of the electrodes becomes irritated, he may depressurize his suit during the flight and remove the electrodes. This is clearly highly undesirable, since it may lead to various unexpected emergency situations.

The problem of electrode placement is of particular importance for the reception of steady information concerning cardiac activity because the movement of the man during the flight may significantly distort the electrocardiogram record.

According to the literature, the contacts most free from interference according to Neb (Matov, 1960) are those from the sternum (Geddes et al., 1960) and from the lateral surfaces of the thorax (Freiman et al., 1960).

The difficulties which have been mentioned above concerning the recording of an electrocardiogram during cosmic flight, bring about the need for special investigations into the designing and testing of various shapes of electrodes and into methods for fastening them so that the most efficient and noise-free record of the electrocardiogram can be obtained over a period of a few days.

Methods

The authors carried out experiments on themselves and on twelve other subjects who were living under normal conditions and wearing ordinary apparel. Tests were also carried out on subjects dressed in space suits and confined for a long time in a mockup model of the capsule of the spaceship. Metallic disks of various thicknesses and diameters were used as electrodes. Various modifications of other more complex electrodes, made up a combination of metallic plates and rubber gaskets to prevent drying of the conducting paste, were also tested.

The electrodes were fastened to the human body by means of small squares of fabric or gauze which were impregnated with various adhesive compounds (Figure 1) or by means of a special system of breast-straps (Figure 2).

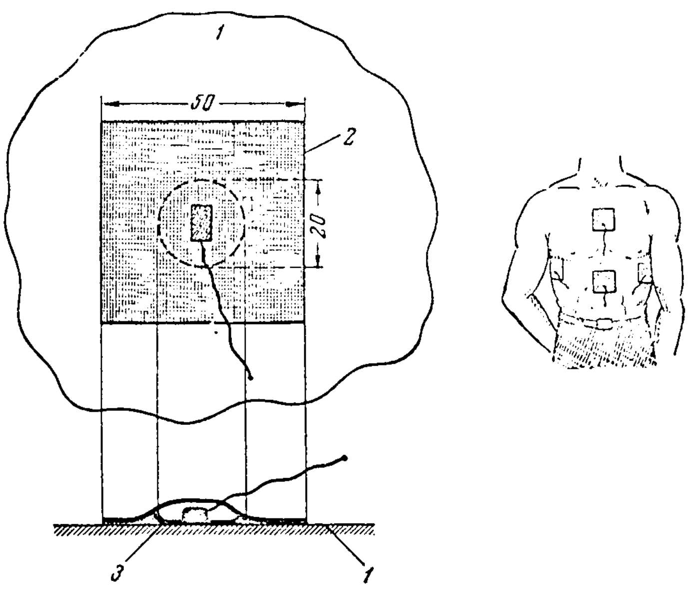


Figure 1. Diagram of the Device and Fastening of the Electrocardiograph Electrodes by Adhesives:

1 - Skin; 2 - Gauze; 3 - Electrode

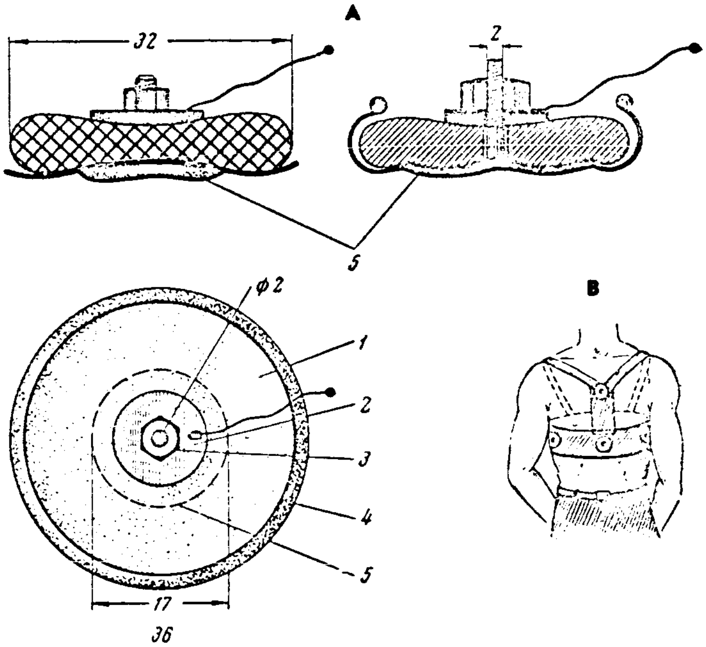


Figure 2. Diagram Showing the Detachable Electrode (A) and the Breast-straps (B) of the ECG Electrodes Placed Over the Thorax:

1 - Sponge-rubber disk; 2 - Washer; 3 - Nut; 4 - Rubber diaphragm; 5 - Electrodes

The reliability of the contact between the electrodes and the human body was checked by repeated measurements of the interelectrode resistance or by comparison with the ECG recordings.

The interelectrode resistance was measured by a testmeter, Ts-52, and by an instrument specially built in our laboratories with which the resistance can be determined with a high degree of accuracy. The electrocardiogram was recorded on a Soviet instrument 4-PFD-7, using an electrocardiograph manufactured by Galileo and Co., and on a special apparatus carried on board the spacecraft, designed for the recording of ECG's during manned space flight.

## Results and Discussion

Of the various leads tested, those most free from interference and and which gave the most information on heart signals were derived from

the thorax. Two leads were selected: horizontal (D-S) and vertical (M-X).[1]

In the vertical position the leads of the electrode are placed on the frontal surfaces of the thorax in the region of the manubrium and the ensisternum, while in the horizontal, on the lateral surfaces of the thorax to the right and left sides of the mid-axillary line at the level of the fifth intercostal space.

Testing of various electrodes showed that least irritation of the skin and most reliable contact was achieved by use of silver disks of 10-20 mm diameter and 0.3-0.5 mm thickness. In order to reduce skin irritation, the edges of the disks should be bent outward. The center of the disk on the side facing the screen should be slightly indented in order to more firmly retain the paste. An elastic screened cable is soldered to the outside of the electrode.

An adhesive, along with gauze or coarse muslin (40 x 40 or 50 x 50 mm) was used to fasten the electrodes onto the skin. The gauze was first glued to the electrode. The electrodes were then fastened onto the skin by an adhesive compound applied to the surface of the gauze. Various adhesive compounds were tested (cleol, collodion, medical glue BF-6, rubber glue, special aviation glue based on nitrocellulose, various commercial types of BF glue, and glue No. 88). The tests showed that the glue, consisting of two-thirds glue 88 and one-third surgical ether, caused the least irritation of the skin and provided the most positive and reliable contact. The adhesive compound should be applied to the gauze in a thin layer since, upon drying, a thin layer is more elastic and less irritating to the skin.

The above system for fastening electrodes was used in the flight of Yu. A. Gagarin and to some extent during the flight of G. S. Titov.

The adhesive requires a long time to dry (up to 30 minutes) and in a number of cases this leads to skin irritation.

In view of this, work was carried out to develop an alternative method for fastening the electrodes. The best results were obtained during tests with the breast-strap system by which the electrodes are fastened in the manner shown in Figure 2. The same electrodes were used as in the previous method. In order to prevent drying of the paste, gaskets made of microcellular rubber with a smooth surface facing the skin

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[1]Use of the D-S and M-X leads for this type of study was proposed by T. I. Akulinichev and R. M. Bayevskiy.

were placed over the electrodes. Gaskets 32-36 mm in diameter were selected, varying in thickness between 5 and 10 mm according to the diameter. The edge of the gaskets facing the skin had a very smooth surface. They were covered with a fine rubber film (to prevent irritation of the skin).

The rubber gaskets were mounted on the electrodes either by means of a small bolt soldered onto the electrode or by a wire shackle soldered onto the electrode and passed through the gasket. The electrodes were fastened to the body by elastic straps (Figure 2). This system of fastening the electrodes was used during the flight of G. S. Titov (Figure 3). Special investigations were carried out to select suitable conducting pastes. These showed that during prolonged experiments the most reliable contact between the electrodes and the skin is obtained when a combination of standard pastes and specially prepared pastes is used.

Figure 3. Attachment of the Electrodes to the Body of the Astronaut G. S. Titov, Using the Elastic-cuff System

Basic components used in the preparation of the special pastes are common salt, glycerol, lanolin, agar-agar and various antiseptic substances. Particularly suitable was the combination of liquid paste rubbed into the skin and a paste of thicker consistency spread onto the electrode. To reduce polarization currents, the silver electrodes used were chlorinated in most cases according to the method proposed by Lykken (1959).

Of considerable interest are the data relating to the changes in interelectrode resistance in the test subjects dressed in normal clothing and remaining in a normal environment compared to that for subjects dressed in a space suit and placed for prolonged periods of time inside the capsule of the spacecraft (Tables 1, 2). Comparison of these data showed that the absolute magnitudes and the regularities in the changes in the interelectrode resistance are in both cases approximately equal (Figure 4). Within the first few days the interelectrode resistance in various subjects was within the range of 5-40 kohm. Within five to seven days the interelectrode resistance increased two- to threefold and within ten to fourteen days it increased five- to sevenfold.

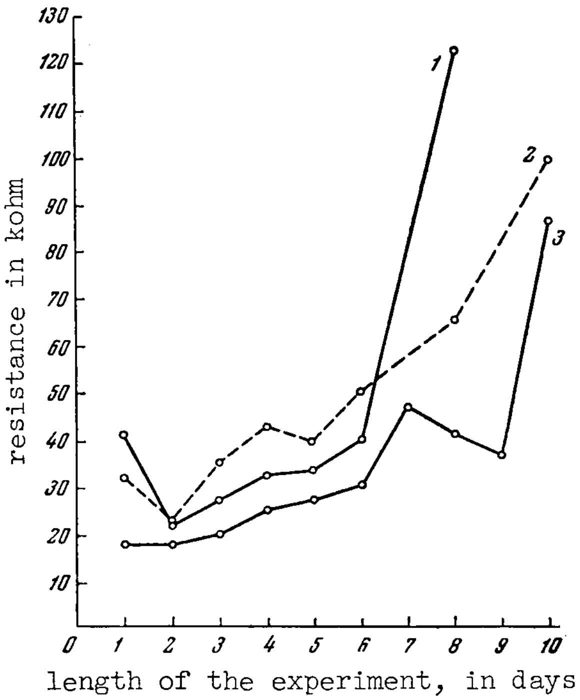


Figure 4. Graph Showing the Change in the Average Values of the Interelectrode Resistance According to Various Methods for Fastening the Electrodes:

1 - D-S (glued); 2 - M-X (glued); 3 - D-S (detachable)

With the two- to threefold increase in interelectrode resistance the amplitude of the ECG peaks, when the spacecraft apparatus was used, was reduced, on an average, by 20 percent and during the five- to sevenfold increase in resistance this amplitude was reduced by 50 percent. No distortion in the form of the ECG was observed during increasing

Table 1. Data Comparing the Change in Interelectrode Resistance for Electrodes Fastened either by a Breast-strap or by Glued Gauze*

| Test No. | Value of magnitude of interelectrode system, in kohm — Days of experiment: 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 7/18 | | —/27 | 23/15 | —/30 | 60/— | | | | | Absence of skin irritation or presence of only insignificant reddening |
| 2 | 8/25 | —/30 | —/40 | 40/20 | 40/— | 65/— | 90/— | | | | |
| 3 | 9/54 | 15/— | 22/— | —/70 | —/30 | | | | | | |
| 4 | 25/35 | —/14 | —/14 | 6/28 | 8/40 | 5/— | 10/— | | | | |
| 5 | 8/65 | 18/— | | 60/— | 100/— | 30/25 | 100/— | —/45 | | | |
| 6 | 5/50 | | 14/— | 10/— | | —/54 | | | | | |
| 7 | 12/15 | 3/— | 6/45 | —/70 | | | | | | | |
| 8 | 11/70 | 6/ | 18/50 | 11/20 | 16/20 | | | 50/— | | | |
| 9 | 20/18 | 4/— | | 20/60 | | | | | | | |
| 10 | 16/23 | 3/11 | 3/38 | | | | | | | | |
| 11 | 9/15 | | —/15 | 12/25 | 10/— | | 60/— | | | | |
| 12 | 30/10 | 28/45 | 14/50 | 13/— | 25/— | | | | 25/35 | | |
| 13 | 12/68 | | 4/35 | | 60/— | | | | | | |
| 14 | 30/15 | —/13 | —/13 | 6/38 | | | 11/— | 19/— | | | |
| 15 | 25/50 | 28/— | 10/— | | | 22/50 | 40/— | 35/75 | 40/100 | | |

*The numerators give the values of the interelectrode resistance when the cuff (breast-strap) system of fastening is used; the denominator values give the same for the glued-gauze fastening.

interelectrode resistance. Study of the ECG records obtained during the flights of Yu. A. Gagarin and G. S. Titov showed that the amplitude of the ECG peaks was substantially unchanged. This allows the conclusion to be drawn that the interelectrode resistance remains substantially unchanged.

Table 2. Changes in the Interelectrode Resistance in Subjects Dressed in a Space Suit and Placed in the Pressurized Capsule

| Interelectrode resistance in kohm when the electrodes were fastened in position | | | | Degree of skin irritation upon removal of electrodes | Remarks |
|---|---|---|---|---|---|
| D-S | | M-X | | | |
| Before | After | Before | After | | |
| | | | | One-day test | |
| 60 | 21 | 33 | 19 | Weak | Fastening by glued gauze |
| 16 | 34 | 10 | 26 | None | Fastening by cuff (breast-strap) system |
| | | | | Three-day test | |
| 21 | 16 | 25 | 8 | Moderate | Fastening by glued gauze |
| | | | | Nine-day test | |
| 12 | 6 | - | - | Moderate | Fastening by breast-strap system |
| | | | | Eleven-day test | |
| 25 | 45 | 22 | - | Moderate | Fastening by glued gauze |
| | | | | Twelve-day test | |
| 14 | 40* | 4 | 500* | None | Fastening by breast-strap system |
| | | | | Thirteen-day test | |
| 9.5 | 32 | 10 | 40 | None | Ditto |

*For 7 days the interelectrode resistance in the D-S lead was equal to 17 kohm and in the M-X lead to 30 kohm.

Thus, as a result of the investigations carried out, two methods of fastening electrodes, the adhesive and the breast-strap, were developed and tested. Both methods insured contact between the electrodes and the human body for 13 days. Within 6-7 days both methods of fastening the electrodes led to a two- to threefold increase in the interelectrode resistance (from 10-20 to 60 kohm). At the same time, the ECG signal decreased by not more than 20 percent. During the following days, the interelectrode resistance in most cases increased rapidly and on the 10th day it was 5-7 times greater than the original value. During this time, the ECG signal decreased by 50 percent.

It should be noted that the breast-strap system of fastening the electrodes is more comfortable and reliable, does not cause irritation of the skin and may be recommended for prolonged experiments.

Summary

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Types of electrodes for recording the electrocardiogram during space flights and methods of attaching them to the body were studied by the authors in experiments carried out on themselves and on 12 volunteers. Silver discs, 10 to 20 mm in diameter and 0.3 to 0.5 mm thick, gave good electrical contact with the least irritation to the skin. The edges were rounded and the undersurface was cupped in order to retain a layer of conducting paste. This consisted generally of sodium chloride, glycerol, lanoline and antiseptics and was best applied as a liquid layer to the skin and as a semi-solid layer to the electrode. The electrodes were attached to the chest either by a covering of gauze which was fixed in position with glue, or by means of an elastic harness. These two methods of fixation were used in the respective flights of Gagarin and Titov. The performance of both methods was assessed by measuring the interelectrode resistance and observing the skin irritation. During the first few days after attachment the interelectrode resistance ranged from 5000 to 40000 ohms; after 10 to 14 days the resistance had risen five- to sevenfold. Both methods caused approximately 50 percent loss in the ECG signal after ten days and provided satisfactory contact with the body for up to 13 days. The gauze method occasionally caused slight irritations which were absent in the case of the breast-strap method.

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